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THE CORONARY CIRCULATION IN THE ISOLATED PERFUSED HEART.

By R. RÖSSLER[1] AND W. PASCUAL[2].

(*From the Physiological Laboratories, Cambridge and Cairo.*)

THE experiments described in the present communication have been performed on isolated hearts of the cat and the rabbit. The object of the research was to study in detail the factors governing the coronary circulation in the isolated heart, and to analyse by means of the hot-wire anemometer the action of various coronary vaso-dilator and vaso-constrictor substances.

The hot-wire method for measuring blood flows has already proved its value, but so far it has been used mainly in experiments on the heart-lung preparation and only for the registration of the blood flow through single large branches of the coronary arteries. The effects of the cardiac contraction and of various drugs upon the circulation through a single perfused branch are not necessarily the same as their action on the entire coronary system when the whole of it is perfused from a common source. In the first case the perfused branch is left in close connection (through arteriole and capillary anastomoses) with those coronary arteries which continue to receive their blood supply from the aorta. In the second case no such collateral connections can vitiate the observations, since the entire coronary system is perfused under identical conditions and the entry of blood into the perfused area from extraneous sources is prevented.

Our experiments are similar to those of Langendorff [1900] who also worked on the isolated heart but used the pressure changes in the coronary cannula as a measure of the coronary perfusion. Langendorff perfused the heart through a cannula introduced into the aorta, a method which is open to considerable criticism. Moreover, his recording system was one of low vibration frequency, so that the results he obtained cannot be accepted without being verified by more modern methods.

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Recently Hochrein and co-workers [1930 and 1931] severely criticized the use of the hot-wire method for blood-flow measurements. They maintain that the hot-wire anemometer is no more suitable for measuring rapid changes of blood flow than a mercury manometer is for measuring rapid changes in pressure. This criticism has been subjected to a detailed analysis by Davis, Littler and Volhard [in Press] who have shown it to be without foundation.

METHOD.

The isolated heart was perfused with oxygenated Ringer solution (NaCl 0·92; KCl 0·042; $CaCl_2$ 0·024; $NaHCO_3$ 0·024 p.c.) through a cannula devised by one of us [see Rössler, 1928]. This cannula has the following advantages. It allows the perfusion fluid to enter only the coronary arteries, it prevents leaks through the aortic valves, and moreover it is not tied to the coronary artery itself. Thus there is no risk of kinking of an artery against the nozzle of the cannula or narrowing of the bore of the artery by the cannula. The filling of the heart through the Thebesian channels was prevented by draining the ventricles of all fluid; leakage was therefore negligible. The reservoir holding the perfusion fluid was stoppered by a hot-wire container 4 mm. in diameter. The deflections of the string galvanometer were proportional to the flow of fluid up to about 35 c.c. a min., so that up to this limit the records obtained needed no correction except for the lag of the hot wire. This lag was of the same order as in the experiments of previous workers, namely about 0·02 sec. The volume of air above the fluid in the reservoir was kept very small and as constant as possible, the reservoir being refilled at short intervals. The perfusion pressure was kept constant at 0·5 cm. H_2O. The connections between the reservoir and the cannula were made of rigid tubes. On the way to the heart the fluid passed through a short metal spiral for the purpose of warming. Great care had to be taken to avoid the formation of gas bubbles in this spiral, since the presence of even small bubbles renders the hot-wire registration valueless. For this reason the perfusion fluid, before being introduced into the reservoir, was saturated with oxygen at a temperature not below 39° C. After oxygenation of the perfusion fluid its hydrogen ion concentration was adjusted to pH 7·6 by addition of phosphate buffers. The fluid escaping from the heart was measured either by means of a syphon recorder devised by one of us [see Rossler, 1926] or by the method of Brodie and Cullis [1911]. The first method was used to measure outflow quantitatively, while the second was used to measure the changes taking place in the average outflow. Both methods provided us with continuous registration of the coronary circulation, and in addition we obtained the detailed analysis given by the hot-wire anemometer.

The pressure changes taking place in the coronary arteries were registered by means of a sensitive optical manometer which was inserted immediately above the orifice of the coronary cannula. The vibration frequency of the membranes used varied in different experiments between 90 and 120. Thus the manometer was quite adequate for its purpose. In every experiment, at frequent intervals, the base lines of the hot wire and of the membrane manometer were verified. In both cases the base line signifies the position of the recording system after the coronary flow has been stopped. In this condition the hot wire registers a zero flow while the membrane manometer registers a maximum pressure, *i.e.* the pressure in the reservoir containing the perfusion fluid.

The registration of the movements of the heart was made as represented in Fig. 1. A light metal rod attached to the apex of the heart (A) was carried over a fulcrum to an ebonite wheel. Each contraction of the heart caused the wheel to move in the direction

of the arrow. By the connection (H) these movements were transmitted to a small mirror (M) reflecting a beam of light into the slot of the photographic camera. In all experiments the movements of the heart were registered with a magnification of nine times. The connections between the heart and the mirror registering its movements were rigid and free play was negligible. Although this method reproduced fairly accurately the time relations between systole and diastole, it is obvious that, like any other method of registering the movements of the beating heart, it could only give an approximate idea of the strength of the contraction.

The preparation of the heart itself was made according to the usual routine. At the end of each experiment methylene blue was injected into the coronary cannula in order to make sure that every part of the heart was uniformly perfused and that no perfusion fluid had penetrated into the cavities of the ventricles directly from the cannula. The drugs used were in every case dissolved in 1 c.c. of the Ringer fluid, the pH of which was readjusted just before the injection. The injections were made into the coronary cannula immediately above the heart.

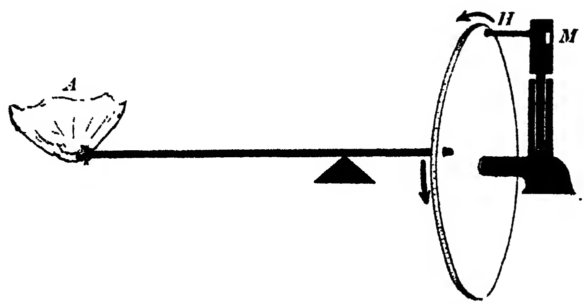

Fig. 1. Apparatus for optical registration of the heart beat.

The effect of systole upon the coronary circulation.

As a result of measurements of pressure changes taking place in the aortic cannula perfusing the heart, Langendorff arrived at conclusions which may be summarized as follows: (a) during the isometric period of contraction, the inflow of blood into the vessels is facilitated and the outflow from the coronary sinus is made possible; (b) during the ejection phase, the inflow is impeded while the outflow is increased; (c) during the diastole, the inflow is facilitated, but there is no outflow on account of the filling of the blood vessels; (d) during the auricular systole, the inflow is increased, but there is no outflow owing to the closure of the coronary sinus. Anrep and Häusler [1928 and 1929] and Häusler [1929], working partly on the whole animal but chiefly on the heart-lung preparation, registered the blood flow in the perfused coronary artery by means of the hot-wire anemometer. They failed to observe any effect of the auricular contraction upon the inflow of blood. Neither did they notice an increase in flow during the period of isometric contraction. They regard the systole of the ventricles as a factor which does not

facilitate the coronary inflow at any stage of its development. The systole acts only as a resistance to the inflow, and this resistance increases as the contraction of the heart becomes stronger. In the case of very strong contractions, a certain amount of blood is regurgitated from the coronary vessels into the perfusing system. During diastole, starting from the dicrotic notch, the emptied coronary system gradually begins to fill up and the inflow reaches a maximum at some period of diastole. The inflow of blood remains at this maximum until the next ventricular contraction sets in. The rate at which the inflow is re-established after systole does not bear any relation to the rate of relaxation of the heart. The refilling of the blood vessels is always slower than the relaxation because the inflowing blood has to overcome the tone of the blood vessels and the viscous properties of the heart muscle. The smaller these two factors are, the more rapid is the refilling.

Hochrein and his co-workers registered the blood flow through the perfused left coronary artery in the whole animal. For this purpose they used simultaneously Broemser's tachograph and the hot-wire anemometer. These observers state that it is impossible to discover an exact relation between the changes taking place in the coronary blood flow and the various phases of the cardiac cycle. Usually they find that the maximum flow occurs during systole; but even in the same experiment and under precisely the same conditions the maximum may shift from the beginning to the end of the systole or even into the diastole. Hochrein regards the contraction of the heart as a factor facilitating the blood flow through the perfused coronary artery. So far as we understand the description of his experiments, the entire length of the connection between the perfusion reservoir and the coronary artery was made of rubber tubing. The introduction of a length of 50 cm. of rubber tubing between two sensitive recording apparatus like Broemser's tachograph and the hot wire is incompatible with accuracy. This probably explains the fact that the two recording apparatus never worked synchronously. The hot wire used in these experiments was of a very low sensitivity and had an enormous lag. Moreover, the records both of the tachograph and of the hot wire show such a quantity of vibrations that in our opinion it is difficult to draw any conclusions at all from the published tracings. Neither calibration nor correction of the records was attempted. In most tracings the base line is not indicated.

Our own experiments on the isolated heart confirm Anrep's observations. The inflow of fluid into the coronary arteries was found to be diminished or stopped during the ventricular contraction. We have never

observed an increased flow during systole. Neither could we confirm Langendorff's observation that the auricular contraction and the beginning of the ventricular systole facilitate the coronary inflow. Examples of records illustrating our observations are given in Fig. 2. Record A of this figure shows a case of a strongly beating heart, the contractions of which were powerful enough to stop the coronary inflow, in spite of the perfusion pressure being 80 cm. Hg. Record B was obtained after an interval of 30 min. when the heart beat had weakened

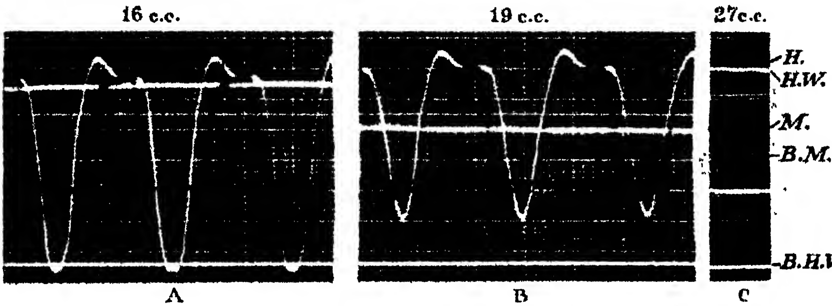

Fig. 2. Effect of systole on the coronary circulation in the isolated cat's heart. The white line *B.H.W.* at the bottom of the record is the base line for the hot wire. The white line *H.W.* showing the deflections is the hot-wire record. The horizontal black line *B.M.* is the base line for the coronary pressure. The black line *M* showing the smaller deflections is the record of the coronary pressure. The black line *H* showing the larger deflections is the optical registration of the heart beat. The upper horizontal white line is the record of the volume flow, obtained by the method of Brodie and Cullis. This line should be disregarded in all tracings, as the flows are given in every case in c.c. per min.; a drop of this line denotes an increase in flow. The time in all tracings is 0·04 sec. All tracings are to be read from left to right. It must be remembered that the coronary pressure and the hot-wire tracing reach their respective base lines when the coronary flow is zero. A deflection upwards means in both cases an augmentation, and a deflection downwards a diminution of the coronary inflow. In C the base line of the manometer is shifted upwards by 4 mm.

somewhat. The coronary inflow was nevertheless considerably reduced during systole but not altogether stopped. Tracing C was taken 1 min. after B when the heart spontaneously started fibrillating. The records obtained by the hot wire are duplicated by the excursions of the optical manometer which shows in A a complete return of the pressure in the coronary arteries to the zero position, denoting a temporary cessation of the coronary inflow; in B the pressure does not reach its base line during systole, showing that some flow took place during the period of contraction. During fibrillation in C the inflow is steady at the level at which

it has been during the second half of diastole while the heart was contracting; this is shown by the hot wire as well as by the pressure registration. In Fig. 2 the hot-wire record is not corrected, which accounts for the fact that the arrest of the flow as registered by the manometer occurs about 0·03 sec. before it is registered by the hot wire. This discrepancy disappears after the necessary correction has been made. The extent of the correction can be seen from the next figure.

Hochrein, in criticizing the use of the hot-wire anemometer for registration of the coronary inflow, states that it registers events with a lag which is as great as 0·1 sec., that it cannot be used for recording changes of flow which are more rapid than about two per second and that it gives records which are completely distorted. The observation of Anrep and Häusler that the inflow into the perfused artery is stopped

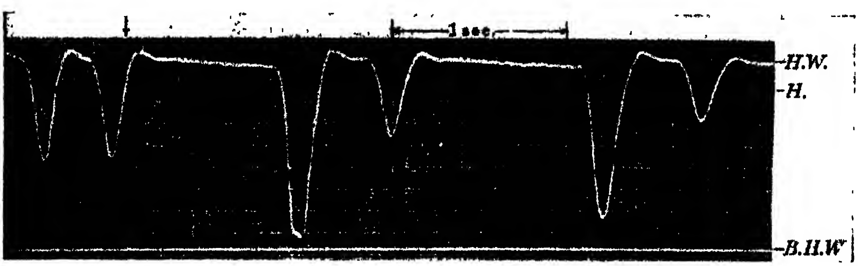

Fig. 3. *H.W.* is the hot-wire record; *H* is the record of the heart beat; *B.H.W.* is the base line for the hot wire. At the arrow the rhythmic stimulation of the auricles was discontinued. The correction of the hot-wire record is shown by the dotted lines.

or diminished during systole is considered by Hochrein to be an error due to the considerable lag and displacement with which the hot-wire anemometer registers changes in flow. According to Hochrein, the flow is diminished during diastole and increased during systole. If Hochrein's statements were correct and Anrep's as well as our own observations are due to such a displacement, this displacement must distort the records enough to shift the events taking place during systole and make them appear in diastole and *vice versa*. This is the only reasoning by which our observations could be explained from Hochrein's point of view. However, if such were the case, a displacement of this kind could happen only as a chance coincidence when the diastole and the systole were approximately equal in length, that is only at a definite heart rate. In actual fact we find that, whatever the heart rate may be, the diminution of the coronary inflow always takes place during systole. We consider that this is conclusively proved by Fig. 3, which shows a

sudden change of the heart rate following the discontinuation of an artificial rhythmic stimulation of the auricle. The length of the cardiac cycle during the artificial rhythm was 0·44 sec. On switching off the electric stimulation, the ventricular contractions became irregular, the successive cycles being 1·1, 0·6, 1·22, 0·56 sec. respectively. In spite of these considerable differences in the length of cycles, and therefore in the relative durations of the respective systole and diastole, we find that in every case it is during the systole that the coronary flow is diminished. It seems impossible to explain records of this type by a lag or by an inaccuracy of the recording system. These observations lead us to the same conclusion as was reached by Davis, Littler and Volhard, who worked on the whole animal and on the heart-lung preparation, and who succeeded in obtaining records of the coronary inflow in hearts beating at the rate of two to five beats a minute. We agree with these observers that Hochrein's hot-wire technique has not been subjected to a sufficiently rigid control.

New method of heart perfusion.

In experiments with coronary perfusion, whether they are made on the whole animal or on the heart-lung preparation or on the isolated heart, the perfusion is usually carried out from a reservoir which is placed at a suitable height and which is connected with the coronary cannula by a system of tubes. This arrangement presents several disadvantages, specially if used in conjunction with a hot-wire anemometer. The friction of the perfusion fluid in the tubes, the ease with which gas bubbles form in these tubes, the necessity of making all connections perfectly rigid are all possible sources of trouble. Furthermore it is necessary to use rather large quantities of the perfusion fluid to fill up the whole system. In order to ensure that our hot-wire records are not affected by any of these possible errors, the new method of heart perfusion shown in Fig. 4 was devised. The Dewar flask *B*, 25 to 100 c.c. in capacity, is filled through the tap *C* with warm oxygenated blood or Ringer fluid. The temperature of the fluid in the flask remains steady during the period of observation. The reservoir *A*, 3 to 5 litres in capacity, is an oxygen pressure chamber. It is connected with an oxygen cylinder and filled with gas at any desired pressure. The hot-wire container is placed between the two reservoirs, and the lower opening of the Dewar flask is connected with the coronary cannula. Since the two reservoirs *A* and *B* have a capacity of 5 litres

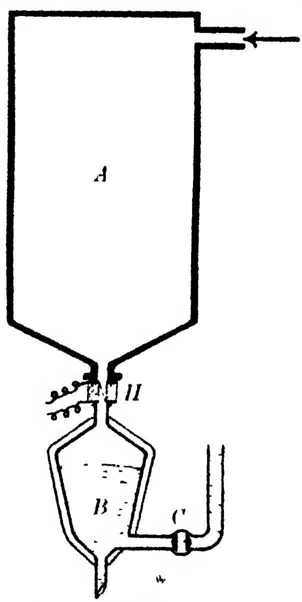

Fig. 4. Apparatus used for hot-wire registration of the coronary circulation in isolated hearts. (Explanation in text

and 50 c.c. respectively, a complete emptying of the reservoir *B* leads to a diminution of the perfusion pressure of not more than 1 p.c. The only precaution that has to be taken with this apparatus is that no oxygen must be allowed to enter the coronary arteries. This method of perfusion obviously eliminates all the above-mentioned disadvantages of perfusion with the usual technique. We also find that hearts perfused in this manner beat considerably better than under other conditions. This is probably due to the higher oxygen tension in the perfusion fluid. The hot-wire records of the coronary inflow obtained with this modified technique were found to be similar in every detail to those obtained with the usual perfusion method. It is therefore clear that, so long as all the possible sources of error are eliminated, the perfusion of the heart through the usual long system of tubes gives accurate registration.

Coronary regurgitation.

On perfusing large branches of coronary arteries, Anrep and Häusler noticed that, when the cardiac contraction is very strong in comparison with the perfusion pressure, a certain amount of blood emerges during systole from the artery and flows back into the perfusion reservoir. The stronger the heart beat the more conspicuous is the regurgitation. Anrep's observations show that in this connection it is immaterial through what channels the perfused area has been filled during the diastole, whether it is from the perfusion reservoir or through various anastomoses from the aorta. The increase of the compressing force in the case of a strong contraction is so rapid that the blood which entered the blood vessels during the preceding diastole has not time to be completely pressed out into the veins. Some of it is thrown back against the perfusion pressure and the arteries thus perform during a strong contraction a rôle similar to that of coronary veins.

It is of interest to find that Drury and Smith [1924] noticed the systolic arrest of the forward flow and the regurgitation by direct microscopic observations of the coronary arteries of the tortoise. Their observations are all the more important, since they were performed on coronary blood vessels which were supplied with blood in the normal way from the aorta.

Hochrein regards the whole phenomenon of regurgitation as the result of an experimental error. Since according to this observer the coronary inflow is maximal during systole, he explains the regurgitation by a direct transmission of the aortic pulse wave through anastomoses into the perfused artery, and not by an active expulsion of blood which filled the perfused area during the preceding diastole. However, in our experiments on the isolated perfused heart, the regurgitation could be observed with the greatest ease. In this case the explanation given by Hochrein cannot apply, because the entire coronary system was per-

fused under identical conditions while the ventricular cavities were kept empty of all fluid. Regurgitation always accompanies a strong contraction, but is especially obvious after injection of some cardiac stimulant such as adrenaline or ephedrine. The lower the perfusion pressure the more easily regurgitation is obtained, but in many experiments we observed it with a perfusion pressure of 100 cm. of H_2O and over. The introduction into the perfusion system of a valve which would permit only a forward flow abolishes the systolic back rush of fluid. The behaviour of the coronary pressure during regurgitation will be described below.

The overshoot.

In the blood-perfused coronary artery of a heart-lung preparation or of a whole animal, the refilling of the coronary blood vessels during diastole is usually slow, the maximal flow being reached only late in diastole. The higher the tone of the blood vessels, the more slowly they are refilled. In Ringer-perfused isolated hearts the refilling is considerably more rapid. In fact it frequently happens that the blood vessels show the smallest resistance to the inflow of fluid immediately after the relaxation of the heart, when they have just been partially emptied by the preceding contraction. Obviously the greater the emptying of the blood vessels, in other words the stronger the contraction of the heart, the greater is the likelihood of this overshoot (see Fig. 5 C and D). Therefore in the isolated perfused heart we find that, in the presence of a systolic regurgitation, the overshoot becomes more conspicuous. Anrep and Häusler have noticed only small overshoots, and these usually towards the end of an experiment or after administration of vaso-dilator substances. Since the overshoot is a purely passive phenomenon which is determined by mechanical factors, it would be incorrect to judge the state of tone of the coronary blood vessels in the perfused heart by the rate of inflow of fluid during the first part of the diastole. It would also be incorrect to do so in the blood-perfused coronary artery of the heart-lung preparation or of the whole animal, but in this case it is because the refilling of the blood vessels is rather slow on account of their tone. It is only by the inflow when it has reached a steady rate that one can judge the state of vaso-constriction or vaso-dilation of the coronary blood vessels. In the first part of the diastole the inflow is either excessively rapid or too slow.

The possibility of the occurrence of what we call the overshoot has been considered already by Porter [1898], who used the idea for the elaboration of his massaging theory of coronary circulation.

The combination of factors affecting the circulation in the isolated heart.

It can be seen from the above description that the coronary circulation in the isolated heart presents a rather complicated picture. During a simple cardiac cycle several changes follow one another with rapid succession, and the volume of fluid passing through the coronary blood vessels is of course determined by the algebraic summation of all these changes. The consideration of these factors is of especial importance in the investigation of the vaso-motor effects of various pharmacological and physiological agents. When faced with the complex series of events taking place in the coronary circulation, it is difficult to see for instance how the vaso-motor action of a drug can be ascertained by simply measuring the amounts of fluid entering or leaving the coronary blood vessels of a perfused heart, unless the vaso-motor effect is very pronounced. The amount of fluid passing through the coronary system per unit of time at a constant perfusion pressure will be determined by the state of the coronary blood vessels on the one hand and by the effect of the cardiac contraction upon the coronary circulation on the other. This is especially the case when drugs are used, since most of the drugs which affect the coronary blood vessels also exert some action upon the heart muscle. These actions may interfere with each other, the vaso-dilator effect for example becoming completely masked or unduly augmented by the cardiac effect. As the following examples show, measurements of volume-flows may in many cases give rise to misleading conclusions, specially in the study of the effect of drugs with weak action or of small concentrations of powerful drugs. In Fig. 5, A and B show the effect of an acute weakening of the heart muscle. As a result of this, the small overshoot disappeared, the systolic restriction of the flow diminished considerably, the coronary circulation increased from 14 c.c. to 21 c.c. a minute, but no vaso-dilation can be detected, for the level of the hot-wire record at the end of diastole in A and during diastole in B is the same. C and D show the effect of a small dose of adrenaline. In the case of weakly beating hearts such a dose changes the flow as it is in B to that in A; that is, the volume flow diminishes on account of a more conspicuous systolic restriction, but shows no sign of a real vaso-motor effect. In strongly beating hearts as in C, the addition of adrenaline brings about still stronger contractions which cause a regurgitation of fluid and an overshoot. During the period of regurgitation, the coronary pressure increases above the base line, showing that the pressure at the orifice of the coronary arteries becomes greater than the perfusion

pressure. The pressure again reaches its base line when the forward coronary flow becomes re-established. The hot-wire tracing in these curves is not corrected, and there is therefore a small discrepancy between the beginning of the forward flow as recorded by the hot wire and by the manometer. After correction of the hot-wire record, the two records coincide. The fact that we deal with regurgitation in the systolic deflection of the hot wire is proved by the behaviour of the coronary pressure and by the fact that this deflection, as has already been mentioned, disappears when a valve is introduced in the perfusion system. Vaso-motor changes are absent as shown by the hot wire, the deflection of which reaches the same level late in diastole in C and D. This is further confirmed by

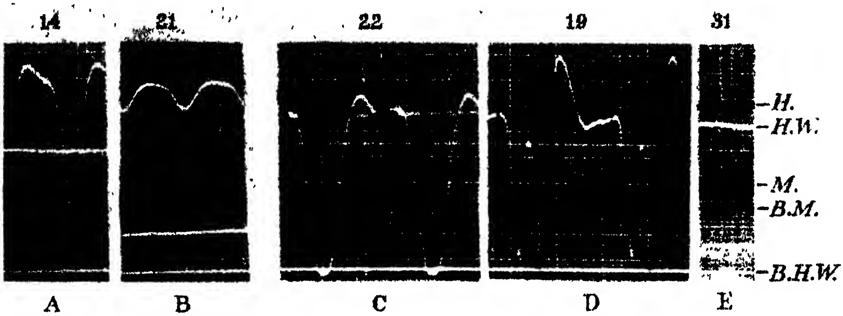

Fig. 5. A and B show the effect, on the coronary flow, of an acute weakening of the heart. C and D show the effect of strengthening of the heart beat after administration of a small dose of adrenaline. E was taken 20 sec. after D and shows the effect of ventricular fibrillation. The lettering is the same as in Fig. 1. The volume flows are given in c.c. per min. (Further explanation in text.)

record E which was taken during fibrillation initiated by a brief faradic current. In this record the hot wire shows a steady deflection within a millimetre of the height reached during the end of diastole in the two preceding tracings. It was ascertained that even this small displacement was only apparent and was due to a shift of the zero position of the hot wire. As regards the volume flow in this particular experiment it was 22 c.c. in C, 19 c.c. in D and 31 c.c. in E. The change in flow observed after administration of adrenaline, in doses which do not exert a vasomotor effect, depends on the balance between the regurgitation and the overshoot. At first one and then the other may predominate, so that the volume flow may show a decrease followed by an increase or *vice versa*. The marked augmentation of the flow in E is the usual effect of fibrillation, and is due to the disappearance of the systolic restrictions.

Fig. 6 shows a number of records obtained on four different hearts. A shows the result of the administration of 0·01 mg. of histamine to the cat's heart, causing a pure vaso-dilation and an augmentation of flow. B indicates the effect of 0·02 mg. of histamine on the rabbit, causing a pure vaso-constriction and a diminution of flow. The same result is obtained by administration of pitressin to both the cat and the rabbit. C illustrates the effect of administration to the cat of 20 c.c. of Ringer solution *p*H 6, together with 0·01 mg. of histamine. The considerable increase in flow is in this case obviously due to two causes, namely to

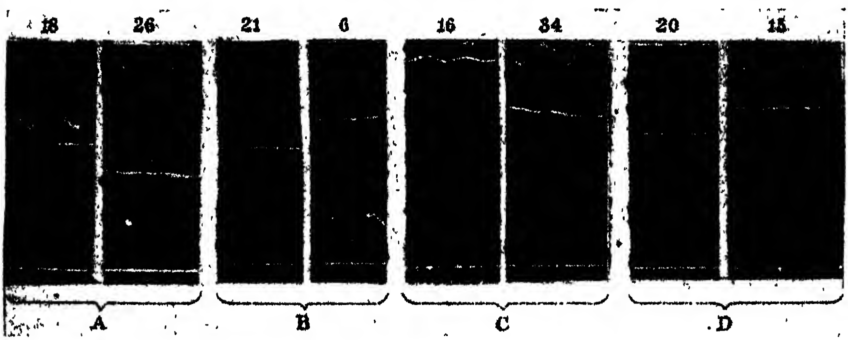

Fig. 6. This shows how the effect of a vaso-motor substance may be distorted by its simultaneous action on the heart muscle. A, effect of histamine 0·01 mg.; cat's heart. B, effect of histamine 0·01 mg.; rabbit's heart. C, effect of histamine 0·01 mg. with a simultaneous weakening of the heart; cat's heart. D, effect of histamine 0·01 mg. with a simultaneous strengthening of the heart; cat's heart. The volume flows are given in c.c. per min. In A from top downwards the lines indicate: manometer, heart, hot wire, flow recorder, base line of the hot wire. The records are easy to follow in the other tracings except that the white line at the top of the first segment of C is the flow recorder. The hot wire is the lower white line which is partially covered by the registration of the heart beat. In the second segment of C the hot-wire registration is at the top. In C and D the base line of the manometer is also given.

considerable vaso-dilation, which is shown by the rise of the diastolic level of the hot-wire record, and to a weakening of the heart which is shown by the diminished effect of systole. Tracing D presents a special interest. It shows the effect of the administration of a small dose of adrenaline together with histamine. In this case, in spite of the obvious vaso-dilation, the coronary flow diminished owing to increased systolic restrictions. Thus we see that the effect of vaso-dilation may be greatly augmented by a coincident weakening of the heart, leading to an excessively large increase in volume flow or conversely, in the case of strengthened contraction, a diminution of flow may take place in spite

of a considerable vaso-dilation. These examples show how difficult it is to make conclusions regarding the nature of various drugs with very weak effect from measurements of volume flows without a more detailed analysis as given by the hot-wire anemometer.

How very involved and complicated the action of a drug can be is shown by the effect of a strong dose of adrenaline, see Fig. 7. A rabbit's heart perfused at 80 c.c. H_2O pressure was made to fibrillate in section A of Fig. 7. The hot-wire deflection shows a straight line, the height of which depends on the degree of relaxation of the coronary blood vessels. In B the heart spontaneously resumed its beat. The height of the hot-wire deflection in the second half of diastole is equal to that observed during the preceding period of fibrillation. The coronary pressure is also the same in both curves. Between B and C 0·3 c.c. of 1 : 40,000 adrenaline (Parke, Davis tabloids) was injected, and soon the heart beat became stronger. The seventh beat produced a complete restriction of the flow during systole and a somewhat increased overshoot. Regurgitation begins from the ninth beat, and this is accompanied by an increased overshoot. Both are seen to grow with every successive contraction, until in D they reach a maximum. The blood vessels are not yet however dilated, as is again shown by the fact that the hot-wire deflection at the very end of each diastole is not higher than in C and B and in A during fibrillation. In D, during periods of regurgitation, the coronary blood-pressure is higher than the perfusion pressure, as can be seen from the fact that it crosses the base line. Only in E do the blood vessels show a considerable dilation, the presystolic level of the hot-wire deflection being considerably higher than before. The regurgitation is, however, still considerable. In F the regurgitation begins to diminish but not the vaso-dilation, in fact the presystolic level of the hot-wire record is even somewhat higher than in F. During all this time the heart rate was controlled at 150 beats per min. In G the artificial rhythm was discontinued, the heart beating now at 94 beats. The regurgitation, which had disappeared during the artificial rhythm, is also absent in G. Simultaneously with the disappearance of the regurgitation, the coronary pressure during systole becomes equal to the perfusion pressure, showing that only an arrest of flow is taking place. The diastolic level of the flow denotes that the considerable dilatation persists. This is further confirmed by sending the ventricle once more into fibrillation as in H. In this last section of Fig. 7, the fibrillation level of the deflection is equal to the presystolic level in G. The debated problem whether the coronary dilatation is due to a direct vaso-motor action of the drug or appears only

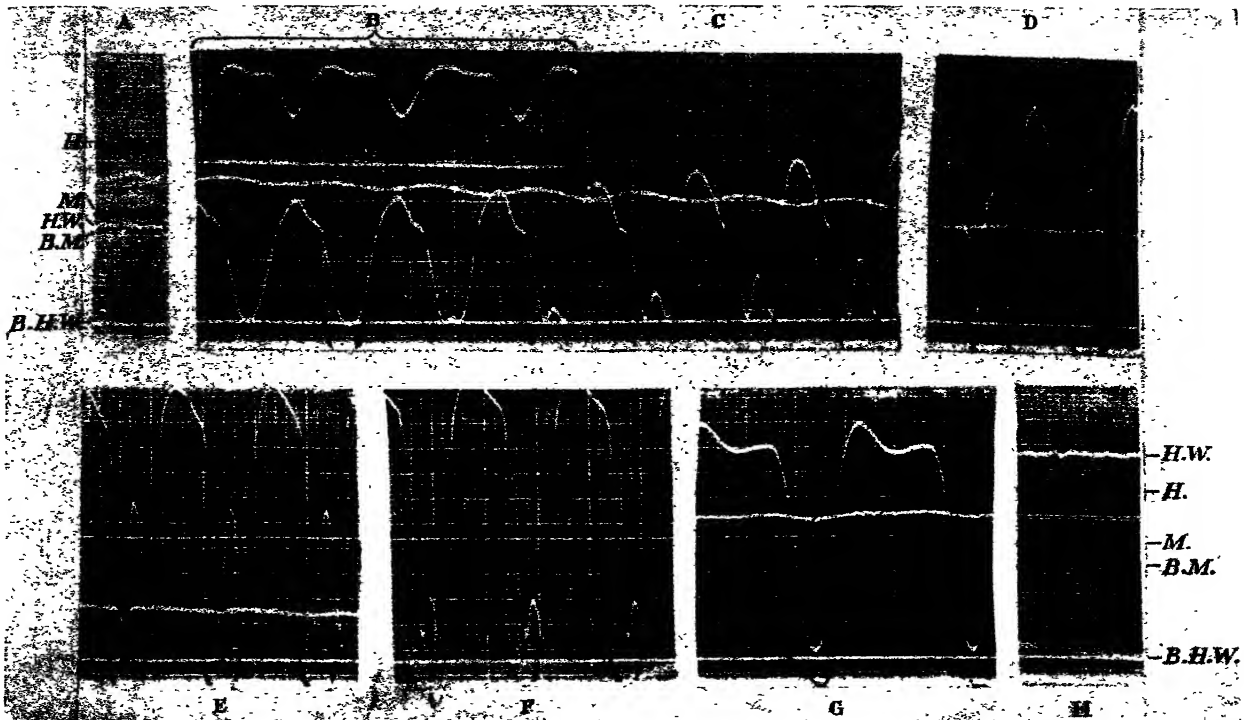

Fig. 7. The effect of a large dose of adrenaline. A, fibrillation before adrenaline. B, resumed beat before adrenaline. C, the seventh to thirteenth heart beat after injection of adrenaline. D, the seventeenth and eighteenth beat. E, 30 sec. after injection. F, 40 sec. after injection. G, 60 sec. after injection. H, fibrillation immediately after G. The dotted line is the base line of the coronary pressure. The rest of the lettering is the same as in Fig. 2. The horizontal white line across the records should not be confused with the hot-wire registration. It is made by the flow recorder. The volume flows per minute in the respective tracings from A to G were: 26, 22, 16, 17. 34, 38, 30 and 39 c.c.

as a secondary effect in response to an accumulation of metabolites is solved by Fig. 8, which was obtained in the same experiment as Fig. 7. The same dose of adrenaline was injected after the heart had stopped in diastole. The vaso-dilator effect is in this case nearly equal to that produced in the beating heart, which can be seen from the extent of the hot-wire deflection. The sequence of events following an injection of adrenaline is usually as described above. In several experiments, however, we noticed that the vaso-dilator effect of adrenaline made its

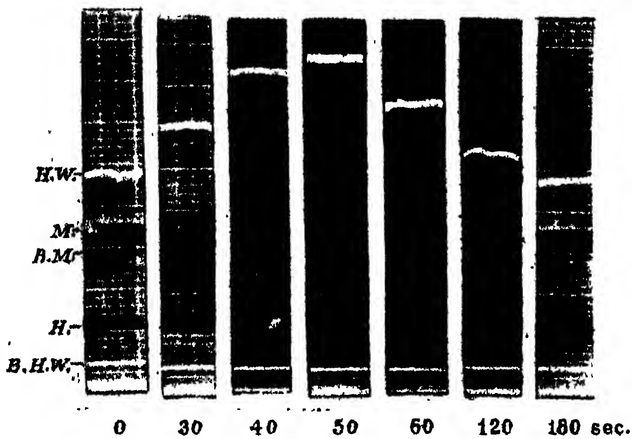

Fig. 8. The effect of adrenaline upon the cat's heart which is not beating. The same dose was used as in the preceding figure. The lettering is the same as in Fig. 2. The first section was taken before administration of the drug. The others were taken after administration at intervals indicated in the figure.

appearance somewhat before the strengthening of the heart beat. In these cases adrenaline causes at first an augmentation of the volume flow which is rapidly succeeded by a diminution, due to the stronger cardiac beat. The subsequent changes are the same as those described for Fig. 7.

We consider that the experiments described above are an example of the most complete analysis of the action of a drug which is possible by the hot-wire anemometer. Without such analysis it is extremely difficult to determine either the mode of action of drugs on the coronary circulation or the minimal dose which is required to produce an action.

SUMMARY.

1. The coronary circulation in the isolated perfused heart is analysed by means of the hot-wire anemometer; the chief peculiarities of this circulation are discussed.

2. A convenient method for optical registration of the heart beat and a new method of heart perfusion are described.

3. The statement of Hochrein and co-workers that the coronary flow in a perfused artery is maximal during systole is not supported by our experiments. The inflow is diminished by the contraction of the heart in proportion to its strength.

4. Certain peculiarities of the coronary circulation such as regurgitation and the overshoot are described and analysed.

5. Examples are given of the action of various drugs, and it is shown how the vaso-motor effect of a drug may be masked, accentuated or reversed by the action which the drug may simultaneously exercise on the heart beat.

We wish to express our sincere thanks to Prof. G. V. Anrep for suggesting the subject of this research and for his constant help, advice and criticism during the work.

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THE LATENT PERIOD OF SKELETAL MUSCLE[1].

By J. ROOS (*Utrecht*).

Part I. Introduction and historical notes.

In the year 1850 Helmholtz found that skeletal muscle does not shorten immediately after the direct application of an instantaneous stimulus, but after a measurable time, which he designated the latent period. This he determined to be 10σ.

After the publication of Helmholtz's work [1850] the latent period was investigated by many physiologists, all of whom found different values for its duration. Studying these results one comes to the conclusion that the latent period recorded has become progressively shorter, as mechanical recording instruments have improved.

The general interest in the problem of latency increased when it was found, that while recording the action current of the muscle the latent period was absent, or seemed to be absent. This discrepancy has contributed in a great degree to the opinion, that contraction and action current are two phenomena which are not in any way connected with each other. They would begin at different times, and end at different times, further they would be independent of one another, and they would therefore originate from different processes in the muscle.

Einthoven [1913, etc.] and his collaborators Hugenholtz [1921], Arbeiter [1920], Kristenson [1928] and others have shown in a series of investigations that in the heart muscle there is an intimate association between these two phenomena, and that one never can be recorded without the other, provided that mechanical registration is performed accurately. Their dependence upon each other was obviously complete, for these investigators could never succeed in separating them by poisoning the heart in any way. Hartree and Hill [1921], Gasser and Hartree [1924] and Fulton [1925 *c*], using quite different methods, came to similar results when working with skeletal muscle. Not only was this relationship established for initial tension and action current, but Gasser and Hartree found in addition, that heat production was

[1] The experiments were made in the Physiological Laboratory of the University of Leiden. Director at that time: Prof. Dr W. Einthoven.

inseparable from these two. Fulton [1925*a*] showed the further interdependence of action current, heat production and tension in fatigue.

One can conclude that at the present time the intimate association between mechanical and electrical phenomena is more or less generally recognized and accepted. But what exactly the relationship is, particularly the time relationship, has not yet been established. When electrogram and mechanogram are recorded simultaneously, it is commonly found that the former is completely or partially registered before there is any evidence of the latter. Both heart and skeletal muscle give this result.

de Jongh [1923] registered simultaneously electro and mechanocardiogram and gave a critical review of the published investigations on this subject. He pointed out that in the frog heart as well as in the heart of the rat the commonly stated time relation between the two phenomena will not be registered, if the mechanical recording instruments are sufficiently sensitive. He came to the conclusion that both phenomena begin at the same time, or at the utmost are separated from one another by some few thousandths of a second. Fulton [1924, 1925*e*], who investigated these time relations on skeletal muscle, came to other conclusions. Even in the muscle preparation with the circulation intact the electrical response never appeared simultaneously with the beginning of mechanical response, but definitely preceded any mechanical alteration. He could therefore not accept de Jongh's conclusion for skeletal muscle, and neither that of Mines [1913], that the electrical response is caused by the same chemical process in the muscle as the contraction, viz. the liberation of lactic acid.

The latency of the mechanical response having become a decisive factor in our understanding of the mechanism of muscular contraction, this problem was selected as the basis of this study. The latent period is furthermore the most suitable for determining the moment of onset of the mechanical alteration. For de Jongh not only succeeded in recording the beginning of the mechanogram as soon as that of the electrogram, but he demonstrated at the same time the possibility of leading the action current in such a way, that it even began after the change of form. Therefore the electrogram is not a suitable object for comparison, if we intend to measure time relations of the mechanogram exactly, and the moment of stimulation is preferred for this design. Some authors practised a correction for the possible delay of the electrogram in order to remove this difficulty; that such a correction is inadequate may be inferred from our results.

A great number of measurements of the latent period may be found in the literature. Some authors used the shortening, others the thickening of the muscle in order to record the mechanical changes. Some of these measurements are presented in Table I.

TABLE I.

Author	Year	Length (L) or thickness (Th) of muscle	Latent period σ	Notes
Helmholtz	1850	L	10	—
Place	1867	L	3·8	—
Gad	1879	L	7·4	—
Von Regeczy	1888	L	2–4	—
Koranyi and Vas	1893	—	0	Microscopic observation
Yeo	1888	Th	5	—
Burdon-Sanderson	1895	Th	3·6	—
Bernstein	1897	Th	4·8	—
Durig	1901	L	2·4	—
Pratt and Eisenberger	1919	Surface	14–20	—
Steinhausen	1921	L	3·6	—
Rauh	1922	L	2·1	—
Judin	1923	L	6	—
Bethe and Happel	1923	L	2·7	Calculated for muscle element
Kleinknecht	1924	L	3·4	—
Fulton	1925	L	2	Muscle with circulation intact

It is remarkable that most authors of the last 35 years agree on this point, that they attribute an absolute meaning to their results: they consider the latent period found to be a physiological phenomenon, viz. as a time occupied by processes that must take place in the muscle before any alteration of form can occur. The older investigators, on the other hand, do not give such definite interpretations to their results, and it is surprising that their work, much of which merits attention, is quoted so seldom in recent literature. A little part of their work, which interests us especially, may shortly be mentioned here.

Helmholtz [1850, 1854] was struck by the fact, that the condition of the muscle and various other factors influenced highly the latency measured. He therefore thought it possible that the energy of the muscle began to rise immediately it was stimulated, but this occurred so gradually that during the first 0·0093 sec. (his latent period) the increase was not more than 1 g., and consequently escaped observation. Place [1867], who on Donders' advice practised isometric contraction, found a latent period half as long as recorded by Helmholtz. Nevertheless he dared not attribute an absolute value to his results, but asked himself whether or not the sensitiveness of the instruments used was responsible for the results in the first place. He too considered the possibility that

stimulation was followed by contraction without any delay, but that the latter could not be recorded at its onset. Neither did Gad [1879] regard his latent period, measured at 7·4σ, as being reliable. In a great number of experiments Tigerstedt [1885] could never find a latency shorter than 4σ. Nevertheless he concludes that mechanical latency of the muscle element must be of the same order as that of the action current. A striking short latency was found by von Regeczy [1888]; nevertheless he designated the 1·9σ he found as a seeming latency. Most interesting are the experiments of Koranyi and Vas [1893], whose work differs considerably from all the others, both in method of investigation as well as in the results obtained. These authors combined the microscopic observation of a muscle fibre of the frog's tongue with observation of the action current. To do this they observed the microscopic preparation and the meniscus of the capillary electrometer through the same hole of a rotating disc which interrupted and closed the primary current of stimulation in the same time. The muscle being tetanized in this way a stroboscopic image was formed from the muscle fibre as well as from the electrometer, so that both images gave the situation at a well-defined moment after the beginning of stimulation. This moment was chosen by adjusting the electrical contact on the disc, enabling the investigators to study both phenomena at different moments of the contraction. The remarkable result was obtained that the curve of electronegativity and the curve of shortening of the anisotropic substance agreed almost completely as to their form. Koranyi and Vas concluded that there is a close connection between the action current and the change of form of the anisotropic substance; and their opinion was that the muscle element has no latency at all. In this excellent work levers with inertia and friction are avoided; secondly the difficulties arising from the muscle substance itself, which will be discussed subsequently are reduced here to a minimum. The important difference between the latency of the muscle as a whole and that of the muscle element is clearly understood by these investigators.

It is a pity that Koranyi and Vas did not record their results photographically. The observations were gathered up in a graph afterwards. We must not forget, however, that many of the curves published have only a very limited value for the reader, as the times given in the text are often difficult to be found in the figures, or cannot be found at all.

PART II. SOME DIFFICULTIES IN MEASURING LATENCY.

Many technical difficulties are encountered in recording mechanical changes of muscle; this is especially so if it is desired to reproduce exactly the beginning of it. Some of these difficulties can be entirely eliminated, but others, although they can be reduced materially by improved technique, cannot be completely avoided, with the result that the beginning of the change of form is registered some time after it actually occurs. These errors in recording the mechanogram are due to:

1. Inertia of the lever used will cause a delay.

2. Friction of the moving parts of the lever presents a force that must be surmounted by the force of the muscle before any change of form can reveal itself. The smaller the velocity of movement, the larger is this friction, and consequently this will cause the greatest error in the results of the initial portion of the contraction, which particularly interests us.

3. Inertia of the muscle. The muscle has a mass which must be moved before the lever can record anything. In order to put this muscle mass into motion such a large amount of energy is required that for this reason alone the sensitiveness of registration of the mechanogram is many hundred thousand times less than that of the action current. Einthoven calculated that the energy required for the registration of an action current by a suitable galvanometer corresponds with that required for raising 1 c.mm. of the heart muscle 10^{-11} mm. Assuming that in our experiments we can register changes of form of the muscle when 1 c.mm. is raised 10^{-3} mm. the energy required is still 10^{8} times as much as that necessary to produce a visible deflection to the string of a good galvanometer.

4. The muscle further possesses the property of extensibility. Contracted parts will therefore extend parts still in rest, and in this way the total length of the muscle may remain unchanged, whereas contraction is already going on. It stands to reason that the non-contractile parts of the muscle such as the connective tissue between the muscle fibres, the elastic tissue and especially the muscle tendons, will effect the end result in the same way as the muscle fibres not in contraction.

It is clear that the effect of the factors mentioned in (4) will be much less when not the length but the thickness of the muscle is recorded. In this way the tendons are eliminated completely, and a part of the muscle in which muscle fibres predominate may be selected for experiment. Furthermore the great extensibility of the inactive muscle parts

near the cross-section chosen for investigation will not only be harmless in so far as the results are concerned, but will to some extent even favour the local change of form. During contraction, however, the extensibility decreases. Gasser and Hill [1924] have shown that, regarding the muscle as a viscous-elastic body, viscosity as well as elasticity increases during contraction. The contracting adjacent muscle parts therefore present an increasing resistance against local change of form. By limiting the contraction to a rather narrow strip of muscle this counter-effect will be reduced, and the local deformation will be facilitated.

If these various sources of error are taken into account, and suitable methods of investigation are adopted, the inevitable delay which occurs before the actual muscle contraction is registered is reduced to its minimum. One must nevertheless remember that even in these circumstances the latent period of the muscle as a whole is measured, and that this latent period on the grounds mentioned will differ essentially from that of the muscle element. Many of the errors of interpretation found in literature are due to a misunderstanding of this difference.

Finally there is the difficulty of reading exactly the curves obtained. Supposing with Einthoven that the muscle force in the first thousandths of a second after the stimulation increases proportionally with the time, the distance travelled by the muscle mass will be proportional to its cube; but even if this distance is proportional to the square of the time it will be very difficult, or impossible, to determine exactly the beginning of the curve recorded by the lever.

Part III. The experiments.

In these experiments the gastrocnemius of the frog was used. Some animals were curarized before, but after it was found that the results were not improved by curarizing this was discarded, and by far the most muscle preparations have been obtained from non-curarized animals. The experiments were made at room temperature.

The change of thickness was selected for registration. The advantages of this method have already been discussed. By stimulating as great a number as possible of muscle fibres in the cross-section used for recording we tried to exaggerate the total thickening. A strong optical magnification of the motion of the lever assisted in making the beginning of the mechanogram visible at an early moment.

We employed Einthoven's wire myograph, in which the principle of the torsion wire is used. de Jongh [1923] gave a complete description of this instrument and only some details will be referred to here.

The lever (T) is in the form of a right-angled triangle, and is constructed of very thin metal. It is placed vertically and is fixed at its right angle to a manganin torsion wire (d). The wire may be turned by two brass heads (S_1 and S_2). The horizontal side of the right angle lies on the muscle, the vertical one is a little shorter and has at its end a small ring, provided with a string, having the thickness of a galvanometer string, in its vertical diameter. This string is placed between two microscopes (M_1 and M_2) and is projected on a photographic plate, after having been magnified 1000 times.

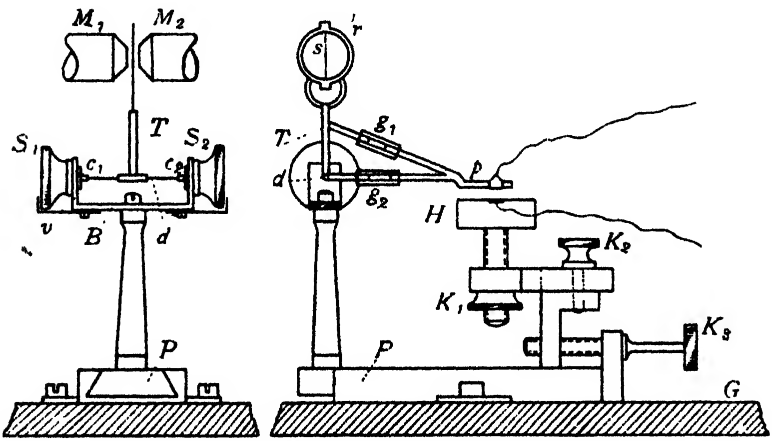

Fig. 1. Einthoven's wire myograph.

The muscle lies on a small ebonite table (H), which can be moved up and down by a screw. For recording it is placed so that the string has a vertical position between the microscopes. If previously the lever was brought in a downward oblique position by turning the heads, the lever compresses the muscle with a force, known by reading the angle of turning on the scale of the heads. Some compression by the lever is wanted, as it has to answer the motion of the muscle surface immediately.

For stimulation a maximal make induction shock was used. The lever was cathode, whereas the platinum anode was sunk in the table. The moment of excitation was signalled on the plate by a string galvanometer, which was placed in a circuit on which the primary circuit for excitation induced a current. In this way the moment of stimulation was recorded without measurable delay.

In Fig. 2 a control deflection of the lever is given. It was caused by a gentle push to its underface. The signal (S) gives the moment of contact. The velocity of the photographic plate is 1000 mm. per sec.

When the muscle lies horizontally on the table in a position perpendicular to the lever, curves are obtained of the form as presented by Fig. 3.

This curve shows a latent period of 4σ, after which the lever develops a sudden almost uniform motion. The deflection rises $33/3{\cdot}5 = 9$ mm. per 2σ, and the optic magnification being 1000 the velocity of the lever was $4\frac{1}{2}\mu$ per σ. This velocity varied in our curves from $4\frac{1}{2}$ to 8μ per σ.

S

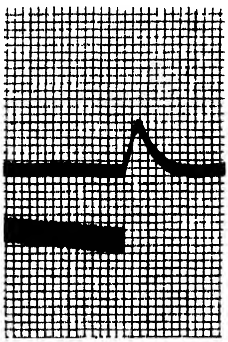

Fig. 2.

S

M

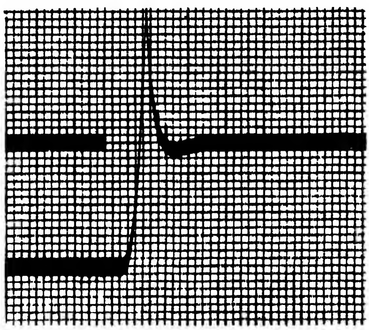

Fig. 3.

Fig. 2. The lever is given a gentle push. S = signal of contact. One division of the abscissa = 1σ. Manganin wire: 0·6 mm. thick.

Fig. 3. The muscle lies horizontally on the table. M = mechanogram, S = signal of stimulation. One division of abscissa = 2σ. Latent period: 4σ.

That the latency found here cannot be the right one, is demonstrated by Fig. 4; here it is seen that also within this time change of muscle form occurs.

Fig. 4 demonstrates that the large deflection is preceded by a small downwards one, with a latent period less than 3σ. When such a negative deflection appears, it is most suitably observed in the first mechanogram recorded by a muscle. When the stimulus is repeated it becomes smaller and smaller, and after a few contractions, say 3–5, it has completely disappeared. The curves reproduced in Fig. 3 and 4 give an example of this; Fig. 3 represents the third mechanogram of a muscle from which Fig. 4 gives the first one. The negative deflection demonstrates one of the difficulties of registering: one should bear in mind the possibility that in those curves where it is not present as a separated deflection it

nevertheless has influence, and manifests itself only by delaying the beginning of the positive one.

The negative deflection, the expression of the decreasing of the muscle

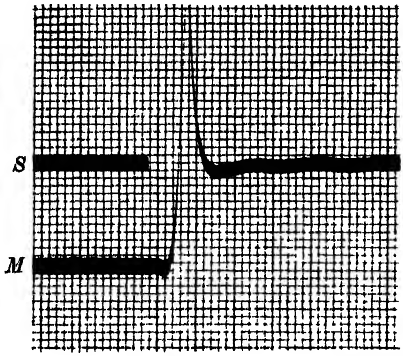

Fig. 4.

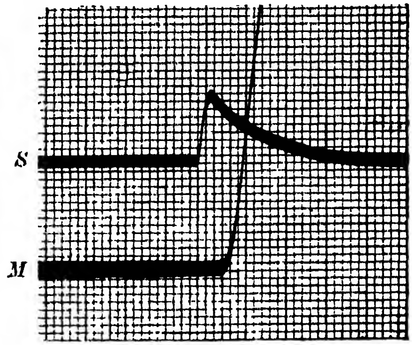

Fig. 5.

Fig. 4. The muscle lies horizontally on the table. M = mechanogram, S = signal of stimulation. One division of abscissa = 2σ. Negative deflection first. Latent period less than 3σ.

Fig. 5. The muscle lies over the narrow table. M = mechanogram, S = signal of stimulation. One division of abscissa = 1σ. Latent period: 3σ.

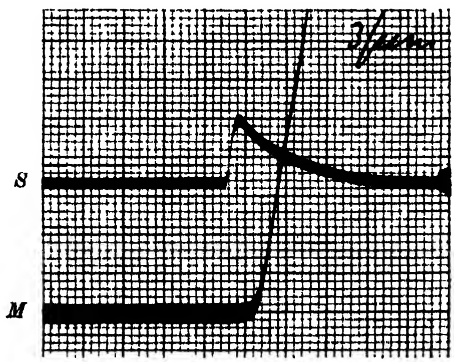

Fig. 6. M = mechanogram, S = signal of stimulation. One division of abscissa = 1σ. Latent period shorter than $\frac{1}{2}\sigma$.

diameter at the spot where the lever lay, could be prevented by using a narrow table, which supported only the middle part of the muscle, its ends hanging downwards along the table. The position of the lever was across the muscle, consequently parallel to the table length. In Fig. 5 a curve is presented, recorded in this way.

As in all the subsequent curves the velocity of the photographic plate was here 1000 mm. per sec. After a latency of 3σ the steep curve begins abruptly. By adjusting the pressure of the lever on the muscle it is shown that this abrupt thickening of muscle is preceded by a very slight one, with a latency which is much shorter than 3σ (see Fig. 6).

Fig. 7 gives the beginning of this original curve, magnified about 4 times.

The curve exhibits a latent period less than $\frac{1}{2}\sigma$. Already at the first ordinate after the stimulus the zero line is left, but before this point the curve is not suitable for exact measurement, the ordinate hindering this. In order to approach as nearly as possible the beginning of the mechanogram another mean had to be devised.

Part IV. The form of the curves.

The curves, one of which is presented in Fig. 7, show two phases, the transition of one into the other being rather abrupt. The initial phase which takes about 3σ, has an average velocity not more than 1/36th of that during the following phase, the latter showing an almost uniform motion.

By measuring this curve and others the motion of the lever during the initial phase is found to be approximately a uniformly accelerated one. The distance (S) travelled by the lever and the corresponding time (t) elapsed since the beginning of the stimulation, measured for different points of the record of Fig. 7, are included in Table II. Both coordinates are given in millimetres, one scale of the ordinate corresponding to 3·5 mm., and 1σ to 3·81 mm. If now acceleration (a) is calculated after the formula $a = 2S/t^2$, we obtain the values for a given in the last column.

Table II.

Point	S	t	a
1	0·06	2·78	0·016
2	0·07	2·97	0·016
3	0·13	4·34	0·014
4	0·27	5·75	0·016
5	0·45	8·05	0·014
6	0·48	8·19	0·014
7	0·77	10·50	0·014
8	1·15	11·80	0·016
9	1·29	12·52	0·016

Point 1 of this table is the first that can be determined exactly by measurement. By constructing the tangent of the curve at this point and the point of intersection of this tangent with the zero line one is able, however, to get more closely to the beginning of the curve. From

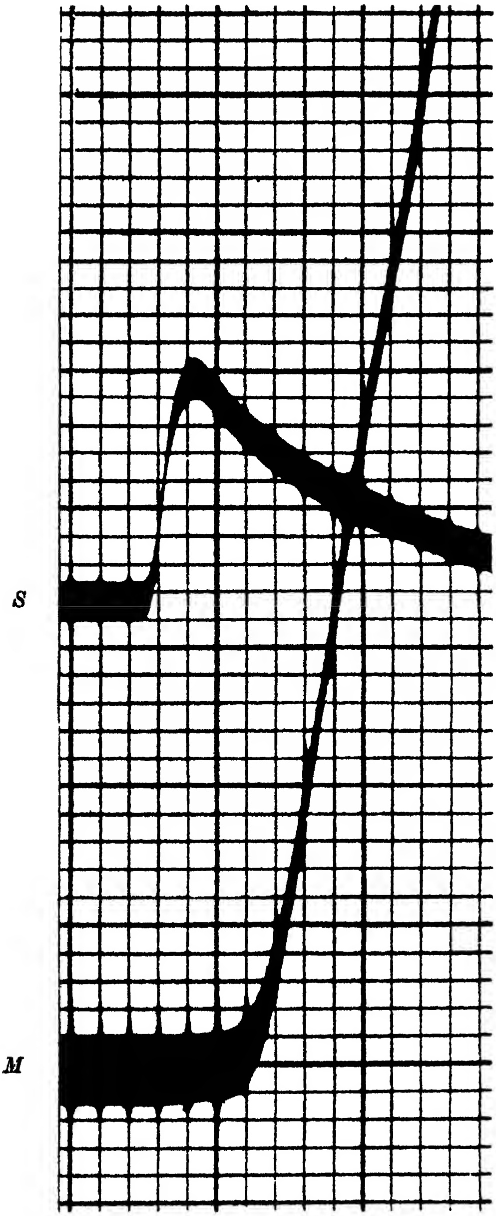

Fig. 7. Magnification of Fig. 6 about 4 times. M = mechanogram, S = signal of stimulation. One division of abscissa = 1 σ. Latent period shorter than $\frac{1}{2}\sigma$.

the form of the curve it can be inferred that this point of intersection is situated after the point where the mechanogram actually commences; and its distance from the beginning of the stimulus being $0{\cdot}4\sigma$ the latent period must be shorter than $0{\cdot}4\sigma$.

A motion of the character such as the one described is often met with in nature. As an example may be mentioned the motion of a falling body under gravity: during the initial part of the fall the velocity is minimal, and consequently air resistance may be neglected: the movement is about uniformly accelerated. As velocity progressively increases the air resistance also increases until equilibrium with gravity is established, and when this has occurred velocity of the body undergoes no further changes but remains constant.

If the curve recorded is the result of a movement under similar circumstances one comes to the following conclusion. During the first few thousandths of a second following stimulation very little resistance is met with by the movement of the torsion lever. After 3σ, however, the resistance has increased to such a degree that it is in equilibrium with the muscle force, and from this moment the movement has become about uniform. If we keep in mind the small rotation of the lever (the complete deflection of the record is the expression of a movement of the muscle surface of not more than 40μ) this change of resistance must be attributed to the internal resistance of the muscle, which consequently would reach its maximum after 3σ.

In this line of thought muscle force is supposed to remain constant during the period discussed. This cannot be proved however. If muscle resistance against mechanical change increases actually in the ratio here described, muscle force must develop very quickly, namely in the first ten-thousandths of a second.

These considerations lead to a conclusion which shows agreement with the results recorded by Gasser and Hill [1924]. These authors studied the internal mechanical conditions of muscle, its viscosity and elasticity, by applying a sudden stretch and by releasing it quickly at various moments during a twitch. The results of both methods of investigation supported each other. If the stretch was applied quite early after the stimulus, an excessively large rise of tension of the isometrically contracting muscle was found, larger than corresponded with the new length; in this case a body was stretched which was considerably less extensible and much more viscous than before. If the muscle was stretched at a later moment, *e.g.* at the point where the external tension had reached its maximum or was already in relaxation, a smaller rise

of tension occurred; consequently at this moment the internal rigidity and viscosity must have partly disappeared. The effect of the stretching was greatest when the stretch started as early as possible after the stimulus. The shortest interval investigated was 5σ, and Gasser and Hill concluded from these experiments that after this period resistance in muscle to mechanical change had reached a maximum as the result of the increase of elasticity and viscosity to their maxima. The agreement on this point of the results of Gasser and Hill, who investigated the tension in the length direction, with our conclusion is interesting.

After the terms of the model given for muscle contraction by these investigators the results of the stretching and the releasing experiments may be explained in this way. Muscle contraction sets in by the production of a fine network of elastic elements, permeated with a viscous fluid. This network has the tendency to strain itself in an analogous way as the fibrin network in a clot of blood, but at the moment that it is formed its fibres are slack. If now muscle is stretched suddenly soon after the stimulus, the network is saved from the necessity of shortening under its own energy; it is stretched passively before becoming taut. Consequently its own shortening increases the external muscle tension excessively.

The time required for the network to become taut under normal conditions is regarded by Gasser and Hill as the time during which contraction fails to increase the tension manifested externally, the latent period. Accepting the results of their work it is improbable that the muscle reaches its great inextensibility just after the latent period. For we have shown that the latent period is much shorter than $2\frac{1}{2}\sigma$, the time found by Burdon-Sanderson, and used by Gasser and Hill for their conclusion.

The question can be put whether perhaps a still greater result of the sudden stretch would have been found if it had been possible to apply it still earlier. We do not, however, think that this is possible, considering the time required, before a sufficient part of the muscle is affected by a contraction wave. Taking 3 m. per sec. for the velocity of propagation of the contraction wave passing over the muscle, after the 3σ of the initial phase 9 mm. on each side of the cathode are in contraction; this contracted portion may be regarded as being sufficient to determine the movement of the lever under the conditions of the experiments. It is acceptable that inextensibility in transverse direction reaches a maximum after this time; and investigating muscle length a few σ more may be found. That in the latter case this could happen considerably sooner,

however, is difficult to understand, since the time necessary for inextensibility to reach a maximum is also determined by the time required by the contraction wave to affect a sufficient part of the total muscle length.

We must conclude therefore that the increase of inextensibility does not reach its maximum noticeably sooner than measured by Gasser and Hill, and that it consequently does not precede the external change of form but coincides with its beginning.

As already quoted Fulton [1925*a*, *b*, *c*, *d*] recently investigated the time relations between the electrical and the mechanical changes of the skeletal muscle of the frog. He registered simultaneously action current and shortening of the gastrocnemius. Many of Fulton's mechanograms exhibited a very slight upward movement during 4–5σ before the abrupt deflection commenced. In this respect Fulton's curves are like ours. I cannot understand, however, why the author excludes this small deflection, recorded as a part of the mechanogram, from the shortening of muscle by saying that the slight upward movement was observed before mechanical shortening began. Furthermore the interval of 2 to 2·2σ between the mechanical and the electrical response was measured by Fulton by taking the small deflection as a part of the mechanogram.

Fulton was of the opinion that the initial phase of the mechanogram could be recorded only by registering muscle length, and that consequently the latent period would be found to be shorter than if muscle thickness had been used for recording. This view is difficult to understand; moreover this has been disproved completely by my results.

Fulton's latent period excites comment. After having subtracted $\frac{1}{2}\sigma$ from the interval measured, for instrumental lag and loss through conduction time for the tension through the inactive tendinous parts of the muscle, $1\frac{1}{2}\sigma$ resulted, which time was designated by Fulton the true latency. This time, however, does not give a true image of the interval between the two phenomena investigated by the author, this being much shorter. Nor can it give the latency. For even the actually true interval between the action current and the mechanical change could not give a reliable image of the latent period, the time between the stimulus and the mechanogram, this period being so short that it must be considerably disfigured by an estimated correction for the possible delay of the action current. On this ground I cannot agree with the suggestion, that latency should be measured from the beginning of the action current until the beginning of the mechanogram. (See Fenn [1925].)

PART V. CONCLUSIONS.

By recording the muscle thickness it was established that the latent period of the muscle as a whole is less than $0{\cdot}4\sigma$. When we keep in mind the circumstances accompanying mechanical registration, which delay its results, and the impossibility of determining the very first beginning of the curve accurately, it is obvious that the period between the stimulus and the change of external mechanical properties of muscle must actually be shorter. Essentially muscular contraction and all other physiological processes require a certain time for development, and as the duration of the latent period of the action current is of the order of about $0{\cdot}1\sigma$ [Jolly, 1911; Erlanger, Gasser and Bishop, 1924] it may be concluded that the electrical and the mechanical changes develop simultaneously.

Different names have been given to the latent period, as it was measured, or to parts of it, indicating it as a period during which mechanical properties of the muscle would remain unaffected by the stimulus; "eigentliche," "wahre," and "true" latency are examples. All these conceptions, however, were due for the greater part to the delay caused by errors in registration. The latent period of the mechanogram does not differ essentially from that of the action current, as it is still regarded by recent authors [Steinhausen, 1929]. It should not take an other place in the physiology of muscle than the latent period of the action current.

Many investigators have studied the relation between the latent period and some other phenomenon of the active muscle: the latent period can never be shorter than the first oscillation of the action current; the latter was estimated to take 6σ [Judin, 1923]; true latency ($1{\cdot}5$–2σ) is as great as the rising phase of the action current or somewhat greater; it appears that the duration of the absolute refractory phase and the true latency are intimately related [Fulton, 1925*e*]. These complicated theories are no longer necessary, and even are contrary to facts. The relation between electrical and mechanical changes of the muscle is a more simple one. As was shown in this paper they develop together; further they finish together [Einthoven, 1925, 1928; van Lawick van Pabst, 1928], and it is impossible to separate them from each other. Consequently we must accept their inseparable solidarity for skeletal muscle on the experimental evidence produced, in the same way as this was in fact taken for granted for the electrocardiogram and heart contraction.

The time relations of the electrogram and the mechanogram are shown diagrammatically in Fig. 8.

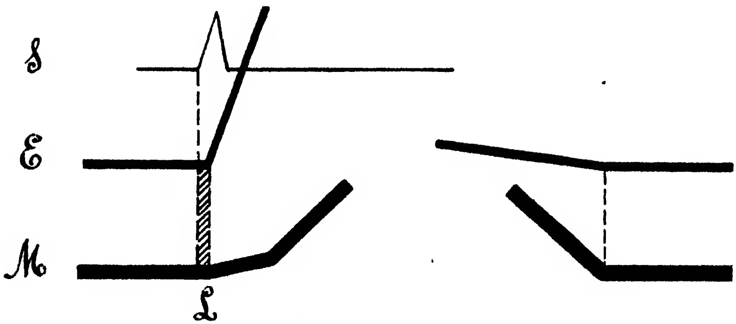

Fig. 8. Diagram of the time relations of the stimulus, electrogram and mechanogram of skeletal muscle. S = stimulus; E = electrogram; M = mechanogram; L = latent period ($0{\cdot}1$–$0{\cdot}4\,\sigma$).

Summary.

The duration of the latent period has become of great importance for our comprehension of muscular contraction and of the relation between the phenomena accompanying it. It was the experimental basis on which rested the theory that electrical and mechanical changes of muscle developed at different times. In our experiments this period was measured, using the gastrocnemius muscle of the frog.

The results were as follows:

1. This time is shorter than $0{\cdot}4\,\sigma$.

2. The view that the electrogram precedes the mechanogram is untenable.

3. The theories that the latent period coincides with a determined part of the action current and with the absolute refractory phase are untenable.

4. It is very likely that action current and mechanogram develop simultaneously.

5. The beginning of the mechanogram exhibits two phases, an initial and a following phase. If their difference is produced by an increasing resistance in muscle against the alteration of form, its time relations correspond with the increase of viscosity and elasticity established by Gasser and Hill. In that case muscle force develops in the first few ten-thousandths of a second.

6. It is not probable that the increase of viscosity and elasticity

found by Gasser and Hill is related to the latent period in the way they suggested.

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THE COURSE OF THE VASO-CONSTRICTOR NERVES TO THE PERIPHERY.

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The course of vaso-constrictor impulses from the sympathetic ganglia to the periphery has not, as yet, satisfactorily been settled. That these impulses travel *via* the sciatic nerve to the frog's web was shown by Roy and Graham Brown [1879], who obtained constriction of the blood vessels therein on stimulation of the sciatic nerve. Jegorow [1892] observed constriction of the peripheral vessels on stimulating the sympathetic nerves when the sciatic nerve was severed. Langley [1911] could not produce vaso-constriction in the frog's web on stimulating either the upper spinal cord or the sympathetic nerves after section of the sciatic, and concluded that the contraction observed by Jegorow must have developed spontaneously.

Trotter and Davies [1909] severed the cutaneous nerves in man and noted that the anæsthetic areas so produced were flushed and hotter than the surrounding innervated skin. They concluded that vaso-constrictor fibres had been severed in the mixed cutaneous nerves and that the loss of vascular tone so produced accounted for the local flushing and rise of temperature. It may be argued that these vascular effects were produced by impulses set up in the degenerating antidromic fibres, for it was noted that the redness disappeared after about 7 days, a time of the same order as that required for the degeneration of these nerves.

Leriche and Policard [1920] observed capillary constriction at the base of the nail on mechanical stimulation of the periarterial plexus. The use of local anæsthetics to block nerve trunks [Lewis and Grant, 1924] produces an evanescent local reddening and rise of temperature in the anæsthetic areas. These authors believed that vaso-constrictor impulses, among others, had been blocked by the nerve anæsthesia.

The widely quoted paper of Langley [1923*b*] contains an element of doubt. After severing all the peripheral nerves of the cat's hind foot, stimulation of the lumbar sympathetic "perhaps caused a trifling pallor but it was too slight to be certain about." Langley concluded that "in all probability" the vaso-constrictor fibres run to the periphery *via* the nerves, and that the peripheral arteries receive small filaments from the nerves, each filament supplying a portion only of the artery.

Papilian and Cruceanu [1924] state that the effects produced by "periarterial sympathectomy" of the carotid of dogs and rabbits differ little, except in the reactions of the pupil, from those produced by removal of the superior cervical ganglion. Wiedhopf [1923] and Schilf [1924], on the contrary, could not obtain evidence for the passage of vaso-constrictor impulses to the periphery *via* the periarterial plexus in the dog and cat. Krogh [1922] stated that constrictor responses in vessels of the frog's web remain after removal of the sciatic from the whole of the thigh, and suggested that the periarterial nerve plexus may be responsible for the survival of these responses.

Woollard [1926] using the anatomical method concluded that the arteries are supplied by nerves in two ways: (1) by fibres forming a plexus around the aorta and continuing along the vessels for some distance towards the periphery, and (2) by additions to that plexus, segment by segment, from nerve trunks. He noted, however, that periarterial sympathectomy caused the degeneration of nerve fibres constituting the plexus at some distance from the seat of injury. Blair and Bingham [1928] followed the degeneration of nerve fibres in the arterial plexus in a human leg amputated some time after periarterial decortication of the femoral artery, and found that degenerating fibres could be traced as far down as the posterior tibial artery. These investigations may be considered to furnish some anatomical basis for the rather prevalent belief that impulses pass for some considerable distance towards the periphery *via* the arterial plexuses.

The experiments described below were undertaken in order to obtain more precise information regarding the exact course taken by vaso-constrictor impulses from the sympathetic cell stations to the minute blood vessels at the periphery.

The intravenous injection of dyes as used by Rous and Gilding [1929] enables changes in the peripheral distribution of blood to be followed, and it appeared probable that this method, combined with nerve stimulation, would yield decisive results. It should be remembered that this method gives no precise information as to which particular

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small vessels are affected, but does bring important information regarding the quantity of blood supplied to a part, which is physiologically at least as important. It would be counter to much well-based work to presume that the arterioles were not largely involved in the changes of blood supply dealt with, and it would seem not unlikely that the other small vessels might also be involved.

METHOD.

The animals were anæsthetized with ether, the right or left stellate ganglion exposed, enclosed in glass-shielded electrodes and stimulated faradically throughout the experiment, *i.e.* until death occurred, using a 2-volt cell and a Palmer's inductorium with the secondary coil at 10 cm. Immediately the stimulation was begun, 3 c.c. per kg. of a 4 p.c. solution of bromo-phenol blue[1] was rapidly injected into the internal saphenous vein. Sixty seconds after the beginning of the injection the abdomen was opened and the abdominal aorta and inferior vena cava were divided, thus rapidly exsanguinating the animals. The vaso-constriction produced was readily manifest by the absence of staining in the ischæmic areas, in marked contrast to the heavily stained neighbouring tissues. The exact distribution in the pelt was more readily seen on the under-surface on account of the dense fur. The time required to shave the skin of the head, neck and forelimbs was sufficiently long to allow some diffusion of dye from the stained skin into unstained areas. Shaving or depilation with barium sulphide was resorted to when small areas of skin were under special examination. A ventral midline incision was made from the point of the chin as far as the abdomen, then an incision at right angles to this was made from the fifth intercostal cartilage to the elbow, thence along the border of the ulna as far as the fifth digit. Labels were stitched to the skin at fixed points such as 1st and 6th intercostal cartilages, 1st and 6th dorsal spines, and both sides of the incision at the elbow. The skin was now rapidly dissected off the forequarters and pinned on to a board, and allowed to dry in air for future comparison. Animals with white forequarters are preferable, as the normal pigmentation of the skin, though distinguishable from the blue stain, nevertheless obscures the result in the dried pelt.

A number of skins were so prepared, and when the areas of vaso-

[1] 400 mg. bromo-phenol blue mixed with 40 mg. NaCl in a mortar and 1·2 c.c. *N*/1 NaOH added and rubbed into a smooth paste. The volume is then made up to 10 c.c. with distilled H_2O. This solution is approximately isotonic and isohydric with mammalian blood.

constriction were noted in each, they were found to be remarkably constant.

RESULTS.

A sharply defined midline, as straight as if drawn with a ruler, was marked out on the skin of the dorsal and ventral surfaces of the head and neck by the stained skin on the unstimulated, in contrast to the unstained stimulated side.

Such a clear demarcation was noteworthy in view of the fact that the blood vessels anastomose freely across the midline. It follows that the arterial plexus described by Woollard [1926] can play no part in the passage of constrictor impulses to the arterioles.

The vaso-constriction extended on the ventral surface as far down as the 1st intercostal space, from here it spread across the chest and axilla, and with the forelimb in the extended and abducted position, to just short of the point of the elbow, thence across the back to between the 1st and 2nd thoracic spines. The space on the upper arm in which vaso-constriction did not occur agreed nicely with the area innervated by the 2nd dorsal segment (intercosto-humeral nerve) in man. The pads were more heavily stained on the stimulated side than on the other, a fact not unforeseen, for Langley [1923*b*] states that the initial pallor, produced by sympathetic stimulation, gives place to flushing of the pads if maintained for more than 45–60 sec.

If there had been no spread of current, and the muscles had remained quiescent (occasionally the poorly insulated leads allowed escape of current to the muscles in the neighbourhood of the stellate ganglion), the muscles of the stimulated side of the head, neck and forelimb were unstained, but careful dissection showed the arteries here, as in the skin, to contain a little of the dye. This observation is at variance with Hartman, Evans, Malachowski and Michalek [1928] who observed microscopically a dilatation and increase in blood flow through muscles on sympathetic stimulation. Hartman, Evans and Walker [1928] observing vessels with the microscope, and Hoskins, Gunning and Berry [1916] using the plethysmograph, have found that small doses of adrenaline increase the circulation through muscles. Compensatory vaso-constriction induced by hæmorrhage was shown by Rous and Gilding [1929] to reduce considerably the blood supply to muscles.

The vessels of the bone marrow were likewise constricted. This confirms the perfusion experiments of Drinker and Drinker [1916] and Drinker, Drinker and Lund [1922]. Compensatory vaso-constriction produced by hæmorrhage while affecting the muscles, among other

tissues, has no effect on the blood flow through the marrow [Rous and Gilding, 1929]. The mucosæ of the eyes, nose and mouth, showed less staining on the stimulated side, that along the hard palate was completely unstained on that side and showed the straight midline observed in the skin.

The thyroid was completely unstained on the stimulated side, the normal side showing by contrast heavy staining. The skull and meninges were unstained on the stimulated side. The brain, however, showed no discernible difference on the two sides. The choroid plexus of the stimulated side was distinctly less stained than the normal, and in one instance was completely unstained.

The submaxillary gland was heavily stained on both sides, and, if anything, more intensely so on the stimulated side. Adrenaline and sympathetic stimulation are known to cause a copious secretion of saliva, and it is possible that the blood flow (in the cat) may be increased. The lymph glands and lymphatics of the neck contained little of the dye on the stimulated side.

The above findings served as controls for subsequent experiments in which, as described below, periarterial decortication, or nerve section, was performed.

The method described was found of little service when such nerves as the femoral or sciatic were stimulated. The mixed nerves gave very inconstant results. This agrees with Langley's observations on mixed nerves [1923*a*].

The lumbar ganglia were isolated and stimulated in some instances. The operative procedure adopted—the transperitoneal route—though speedy, was sufficient to produce a patchy distribution of dye in unstimulated skin. This complication makes observation of the distribution of the nervous impulses difficult, but it is, of course, a difficulty inherent in the operative procedure, no matter what method is used to assess the results.

Periarterial "sympathectomy."

The axillary artery was exposed, care being taken not to injure the brachial plexus; the arterial sheath was lifted up with fine forceps and cut off with scissors until the media was bared all round, for a distance of 1 cm. In two experiments the posterior circumflex artery was also stripped. The bared vessel was then swabbed three times with absolute alcohol, protecting the underlying nerves the while, the alcohol being allowed to evaporate between each swabbing. This drastic treatment hardened the artery; it became supple again, however, when replaced in

the wound. The stellate ganglion was now exposed and stimulated, and the bromo-phenol blue injected.

The areas of vaso-constriction differed not at all from those in the control animals. That the absence of staining below the axillary region was not due to a localized contraction of the traumatized artery was proved by the staining of the pads.

These results were obtained mainly in the cat; the one experiment performed on the dog confirmed the findings in the cat.

Peripheral nerve section.

The median and internal cutaneous nerves (which run together through the axilla) were divided. The stimulation of the stellate ganglion and dye injection showed the absence of vaso-constriction in an area continuous with that innervated by the 2nd dorsal nerve in the axilla, and down the palmar aspect of the forelimb and forefoot. In the middle of the limb this vaso-dilatation encroached upon the extensor surface on both the radial and ulnar margins. Elsewhere the vaso-constriction of the skin was as in the control animals.

The median nerve was severed just before it enters the condylar foramen. This was similarly shown to prohibit the passage of vaso-constrictor impulses to the skin on the palmar aspect of the wrist and forefoot, to the whole of the radial side of a line from the pisiform pad to the middle of the ring finger; the staining encroached on the extensor side of the very tips of the digits but did not completely encircle the claws. The flexor group of muscles, except the flexor carpi ulnaris and the ulnar half of flexor profundus digitorum, were stained. The latter muscle provided a striking example of vaso-dilatation of the radial half, side by side with the vaso-constriction of the ulnar half of the muscle.

The ulnar nerve was cut at the elbow and thereafter the stellate was stimulated and the dye injected. An area of skin on the extensor and palmar surfaces just proximal to the head of the ulna, and down to, and including, the 5th digit and ulnar half of the 4th digit was well stained (see Fig. 1[1]). The extensor muscles and the median group of flexor muscles were unstained. The flexor carpi ulnaris, the ulnar half of profundus digitorum and the m. interossei were well stained.

The cutaneous branch of the musculo-spiral nerve in the cat divides into three fasciculi as it passes over the extensor surface of the base of

[1] The colour of the blue dye does not stand out well in photographs. In the experiments which were the subjects of figures, a 4 p.c. solution of phenol red was injected.

the 2nd metacarpal bone. Experiments were done in which one or other of these minute sensory nerves was cut prior to the sympathetic stimulation and dye injection. These minute nerves are accompanied by fine vessels, which were carefully avoided when severing the nerve. Section of the first branch produced staining of the skin of the radial side of the 2nd digit: section of the second branch showed staining of the opposing

Fig. 1.

surfaces of the 2nd and 3rd digits: and finally, section of the third branch abolished the sympathetic vaso-constrictor impulses to the opposing surfaces of the 3rd and 4th digits. This latter condition is shown in Fig. 2. These results were due definitely to the nerve section and not to effects produced by injury to the skin: this was clearly shown in each experiment by the complete absence of staining in and around the minute skin incisions, a zone of unstained skin varying from ¼ in. to ½ in. was seen between the incision and the beginning of the stained areas.

When the incisions were in areas affected by the sympathetic stimulation it was noticed in every experiment that no dye got into the margins of the wound. It is known [Lewis, 1926] that adrenaline locally applied, even in strong concentration, does not abolish the local reaction either to histamine or *H* substance. A probable explanation of this difference is that in the experiments reported here, the sympathetic

Fig. 2.

stimulation contracted vessels central to those that are affected by the local response to injury, thus withholding the stain from the minute vessels. This point was not further investigated.

If the abolition of the primary pallor in the pads is due to the production of a metabolite—as assumed by Langley [1923*b*]—then this chemical substance, unlike *H* substance, is able to overcome the vaso-constriction of all the vessels supplying blood to the pads.

Conclusion.

The foregoing experiments demonstrate beyond doubt that vaso-constrictor impulses travel to the tissues through the motor and/or sensory nerves.

Evidence is presented of sympathetic vaso-constrictor impulses through the motor nerve to muscles. The clear-cut demonstration of vaso-constriction in one-half of a muscle, viz. m. profundis digitorum, on severing the nerve to the opposite half (median or ulnar as the case may be) is conclusive. If, however, the muscle itself contracts, the vaso-constriction gives way to vaso-dilatation.

The observations (*a*) that vaso-constrictor impulses stop short precisely at the midline of the body, arterial anastomoses notwithstanding, and (*b*) that such small areas of skin as that covering half a cat's digit are deprived of sympathetic innervation by section of the local minute sensory nerve, prove that constrictor impulses do not travel, even for such small distances, in the arterial plexus.

Summary.

1. A method for following the distribution of the sympathetic nerves (and incidentally the motor and sensory nerves) to the tissues is described.

2. The sympathetic nerves follow the motor or sensory nerves to the periphery, branching from them in the vicinity of the minute blood vessels innervated.

3. The periarterial nerve plexus does not conduct impulses to the periphery.

4. Stimulation of the stellate ganglion causes vaso-constriction in the skin, mucous membranes, muscles, bone, bone marrow, meninges, choroid plexus and thyroid.

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OBSERVATIONS ON THE TIME TAKEN FOR CORPUSCLES TO TRAVERSE THE LIVER.

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The view that the liver can act as a store of blood from which that fluid can be diverted to other parts of the body has been urged by a number of workers, either by implication or as the result of specific experiments. Krogh [1912] put forward a plea for such a store, somewhere in the portal system. Mautner and Pick [1929] showed the presence of a sphincter in the hepatic vein which was constricted by histamine; its relaxation allowed the blood from the liver to drain into the general circulation. Poulsson and Dale [1931] showed that this sphincter is released by adrenaline. This apparently is present in some animals, *e.g.* the dog, but not in others, *e.g.* the goat and cat. Recently Grab, Janssen and Rein [1929] have shown by direct methods that the injection of adrenaline produces an immediate expulsion of blood from the liver of between 26 and 59 p.c. of the weight of the organ itself.

The question naturally arises, is there any store of stagnant blood in the liver such as is contained in the spleen, and to a less extent in cyanosed skin? To answer this question essentially the same method has been used as described in the previous paper by Barcroft, Benatt, Greeson and Nisimaru [1931]; a dose of carbon monoxide is suddenly injected into the trachea, and its appearance in, and disappearance from, the small vessels of the liver is compared with the same in the general circulation. The only previous observations on this point were by J. and H. Barcroft [1923], who showed that whereas rats killed after some minutes' exposure to atmospheres which contained carbon monoxide had but little COHb in their spleen pulp, they had as much CO hæmoglobin in the blood of the liver as in the general circulation.

METHOD.

The animals used were cats and dogs; an anæsthetic usually C.E. mixture was given. A cannula was inserted into the trachea and another

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into the carotid artery. An opening was made in the abdominal wall, giving access to the surface of the liver. Samples of blood were taken, when required, from the cannula in the femoral artery and from the liver. The latter were obtained thus: a small tear was made in the surface with a blunt instrument, so that the blood which welled up should come from the most fragile vessels, presumably the "capillaries." Such blood was collected in a 20 c.mm. pipette (in which was a trace of heparine) and transferred to 0·5 c.c. ammonia (2 c.c. strong ammonia per litre). The estimations were made by the Hartridge reversion spectroscope. In one or two experiments it proved possible to obtain more blood; the determinations were then made on samples, each of 0·1 c.c., with a modification of the van Slyke method worked out by one of us (Ray).

After samples of blood had been taken from the general circulation, and from the liver, a quantity of carbon monoxide was injected from a syringe down the trachea and washed into the lung with a syringe full of air. The tracheal tube was occluded before the insertion of the needle into the trachea and remained so for perhaps 10–15 sec., then the cat was allowed to breathe freely. Usually the tracheal tube was fitted with valves and the cat breathed oxygen from a bag fitted to the inspiratory valve. The quantity of CO given differed somewhat in different experiments; usually it was roughly 10 c.c. per kg. weight of cat. Blood samples were taken at suitable intervals from the liver and general circulation.

The technique used for dogs was essentially the same as for cats.

Results.

I. *Cats.* Of eleven experiments performed the most typical was that shown in Fig. 1. In it:

(1) The carboxyhæmoglobin had reached its maximum value in the blood both of the femoral artery and the general circulation within 2 min. of the injection.

(2) No difference could be observed in the percentage saturation with CO as between the arterial blood and that taken from the scratched surface of the liver.

In exceptional cases there seemed to be some evidence that the blood from the liver took a little longer to attain its maximum content of CO than that in general circulation, but we never obtained any certain reason for supposing that the blood in the liver retained its CO longer than in the general circulation. There was no obvious crossing of the curves such as is shown in the skin [Barcroft, Benatt, Greeson and

Nisimaru, 1931] or in the spleen [Barcroft and Barcroft, 1923]. Moreover the cases in which the liver blood seemed to acquire CO less rapidly than the blood in the carotid artery or femoral vein, were those in which the liver was pallid or bloodless, not those in which it was red and turgid.

In one experiment we compared the CO saturation of the blood in (1) the femoral artery, (2) the portal vein, (3) the hepatic vein, (4) the

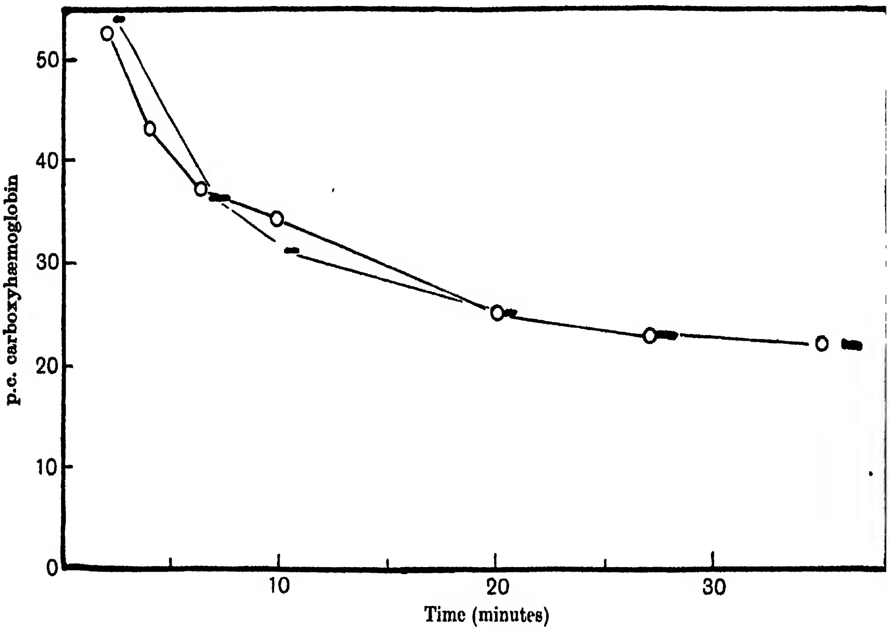

Fig. 1. Percentage of COHb in blood of cat after an injection of CO into the trachea. o = arterial blood; —— = blood from parenchyma of liver. The length of the line indicates time taken for the collection of the sample.

liver. They were all within 3 p.c. of 34–35 p.c. saturation, which is about the experimental error of the method.

Time of drawing sample in min. from giving CO	20	21	22	23	24
Place from which blood was taken	Femoral artery	Liver	Portal vein	Hepatic vein	Femoral artery
P.c. saturation with CO	31	38	35	36	37

It may be urged that our method of taking samples did not tap the actual blood from the vessels in the liver parenchyma. In answer to this we would say:

(1) The blood was often of a hæmoglobin value which differed con-

sideraɒı, ... the circulatory blood [cf. also Barcroft and Poole, 1927]. To give an example:

Time of observation	Grams of Hb per 100 c.c. blood		
	Arterial	Liver	Portal vein
10.26	8·3	—	—
10.34	—	9·3	—
11.5	—	—	7·7
11.15	8·5	—	7·6
11.49	—	8·9	—

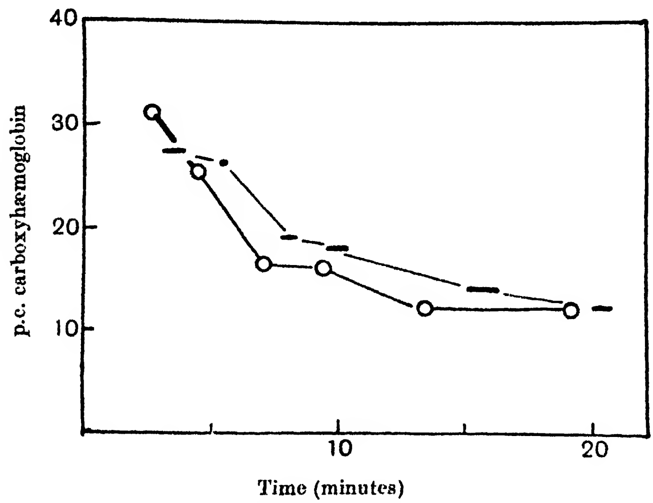

Fig. 2. Percentage of carboxyhæmoglobin in the blood of the dog, after injection of CO into the trachea. ○ = arterial blood; ▬ = blood from parenchyma of liver.

(2) The same method applied to the spleen shows up quite distinctly the difference between the splenic blood and that in the general circulation.

Time of sample measured in min. from administration of CO	30	31	32	32
Place from which blood was taken	Arterial	Liver	Arterial	Spleen
P.c. saturation with CO	25	27	27	11

The above results obtained by the Hartridge reversion spectroscope were confirmed by a modification of the van Slyke technique worked out by one of us (Ray).

II. *Dogs*. Two experiments have been performed on dogs. Fig. 2 shows the result which was typical of both. The dog in this case weighed 11 kg.; it was given morphia and C.E. mixture. Fifty c.c. of carbon monoxide were injected into the trachea; the animal then breathed

oxygen which was led through the anæsthetic and Barcroft. [illegible]ory valve. The result was a little different from that usually obtained with cats, inasmuch as there seemed to be a short but sensible lag, as between the CO contents of the arterial blood and the liver blood. This lag amounted perhaps to 2 min. Such a lag might be looked for with greater confidence in the dog than in the cat on the ground of Mautner and Pick's and of Poulsson and Dale's demonstration of the venous sluice mechanism in the dog. When, however, consideration is given to the fact that the major part of the blood reaching the hepatic vein passes through two sets of "capillaries," it cannot be claimed that even a lag of 2 min. is sufficient to argue any real stagnation--stagnation that is to say, in the sense that the corpuscles are diverted out of the ordinary current of the circulation.

Summary.

1. The time spent by a red corpuscle in traversing the liver is small relative to that which may be spent in the spleen or the skin.

2. Although the liver is a store in the sense that it contains large quantities of blood which can be transferred to some other site, it is not a store in the sense that blood is out of the circulation.

3. The above conclusions correspond with the structures of the liver, the skin and the spleen respectively. In the vessels of the liver there are no considerable diverticula from the general current in which blood can lie. The spleen pulp and sub-papillary venous plexus provide situations aside from the general stream.

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STUDIES ON THE PHYSIOLOGY OF REPRODUCTION.

I. The effect of thymectomy and of season on the age and weight at puberty in the female rat.

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Introduction.

The work of Calzolari in 1898 demonstrated that castration delays the involution of the thymus which normally takes place at puberty. His observations have been confirmed by nearly all of a long line of investigators who have repeated the experiment. This finding naturally suggested the possibility of a reciprocal relationship of some kind between the thymus and testes, and many attempts have been made to demonstrate some change in the gonads as the result of thymectomy. There have been reports of precocious development, of delayed development and of no change at all in the ovaries and testes following thymectomy, but in each instance the data have been too scanty to permit of statistical analysis. In most of the experiments in which delayed sexual development was reported, the animals were underweight or suffered from infections. A more detailed analysis of the literature is being published elsewhere by the author and will not be repeated here. The frequently repeated statement that thymectomy hastens sexual maturity, together with a dearth of careful protocols, led to the present undertaking.

The recent knowledge that the vagina of the rat opens on the day of first œstrus in most cases offers a criterion for the age of puberty. It seemed that a series of rats that was long enough to admit of statistical analysis of the age and weight at puberty, and in which the animals were thymectomized long before maturity, controlled as to diet, and observed as to growth and general condition, should provide a definite answer to the question. The present work is an attempt to meet these conditions and to give a final answer as to whether thymectomy in the young animal affects the age of puberty. The data on the female rats are presented here, and those on the male rats will appear in a later paper.

The experiment was first carried out with rats born during October, November and December, 1929. The results were indecisive, and statistical analysis revealed the fact that there were too few animals in view of the wide variation in the age and weight at which the vagina opened. As the answer to the problem was in these terms, it was considered advisable to repeat the experiment with as many rats as the equipment and the supply of animals allowed. The second series included rats that were born during April and May, 1930. The records for the two series were kept and analysed separately. The diet and care of the animals were the same in both cases. The first experience pointed out the necessity for uniformity of age at operation, and for weighing the rats on the actual day of the opening of the vagina. These corrections were made in the records of the second series. In other respects the second experiment was a replica of the first.

Female rats, comprising a shorter series, were thymectomized in the course of another investigation during the period of December, 1930, January and February, 1931. With greater experience in technique it was possible to thymectomize these rats at the age of 1 day. The age at the opening of the vagina was noted in this series for comparison with the earlier two, but the animals were not autopsied and no data as to completeness of extirpation or weights of organs are available. The diet and care were the same as for the previous experiments, with the exception that about 0·3 p.c. of dried yeast was added to the diet. The number of rats in each series and the operative mortality, as calculated from the rats dying within 24 hours of the operation, are given in Table I.

TABLE I. The number of litters and animals used.

	Rats reaching maturity				Died						
	Females		Males		Died within 24 hrs. after operation	Died between 2 and 60 days			Operative mortality p.c.	Totals	
	Operated	Control	Operated	Control		Operated	Control	Discarded		Litters	Rats
Series I	29	26	16	14	16	0	0	17	35·6	17	118
Series II	39	40	47	47	16	1	3	0	18·0	25	193
Series III	21	19	17	14	17	17	18	4	23·6	20	127
Totals	89	85	80	75	49	18	21	21	—	62	438

TECHNIQUE.

Series I. Winter, 1929–1930.

The rats were obtained from two sources: 36 of the 55 females and 23 of the 30 males were of a standard breed of albinos; the remainder

were of varied breeds. There were no differences in the results in the two groups in this series.

Litters of rats were obtained from the dealer at the age of 2 weeks. The diet consisted of whole wheat flour 67·5 p.c., casein 15 p.c., powdered milk (Klim) 10 p.c., butter 5 p.c., NaCl 1 p.c., $CaCO_3$ 1·5 p.c., with fresh milk and lettuce and (for the mothers of young litters) a little raw meat. The first litters used were operated on during the 3rd week, but the operative mortality was so high that subsequently the operation was done during the 4th week. The rats of each litter were marked by ear punches, weighed and paired by sex and weight as far as possible. The thymus was removed by the method described by Pappenheimer [1914]. The operation was performed under ether anæsthesia and with aseptic precautions. The rats were returned to their mothers after the operation, and were not weaned for at least a week afterwards. Most of the controls of the animals which died during the operation were discarded. During the 5th week the litters were weaned and the males separated from the females. In all cases litter mates of the same sex were kept in the same cage. All the rats were weighed weekly. There were no deaths except operative ones. The females were examined daily for the opening of the vagina. After it opened vaginal smears were made and examined under the microscope, and the quantity of various elements charted. The method was the standard one first described by Long and Evans [1922]. Unfortunately the weight on the actual day of the opening of the vagina was not noted, but the weekly weighing nearest that day was used as the weight at puberty. This was never more than 4 days from the correct day.

The rats were kept in this way until at least three regular œstrous cycles were recorded for each of the rats of any given litter. Four operated animals and their controls were used for a mating experiment. As soon as all of the animals of any of the remaining litters had run through at least three regular normal cycles that litter was killed. The autopsy records included: the weight and age of the animal; the weight of the thymus in the control animals; inspection of the thymus area in the operated animals for remnants of thymus; the weight of the spleen and adrenal dissected clean and weighed in a closed weighing bottle to 0·5 mg. The entire neck and chest organs, *en masse*, were removed and were kept for serial section; the hypophysis, genital organs, spleen and portions of pancreas and liver were preserved for microscopic study.

The observations on the male rats are reported in another paper.

Series II. Spring, 1930.

The experiment provided by the first series enabled us to perform the operation successfully on the 21st or 22nd day of life in each case in the second series. The animals were weaned on the 28th or 29th day. The age and weight on the actual day of the opening of the vagina were noted. The rats were all albinos and except for five litters were all obtained from the same source as Series I. The diet and care were as before. Fifteen male and 23 female rats were kept for a mating experiment. This experiment and those on the remaining male rats will be reported later. The remaining female rats were examined daily as to vaginal smear and later were autopsied in the same way as Series I. Two stitch abscesses were found at autopsy but in no case was a wound infection the cause of death. The thyroid was added to the list of organs which were weighed.

Series III. Winter and Spring, 1930.

During the course of another experiment a group of rats were thymectomized and allowed to grow up. The age at the opening of the vagina was noted incidentally, to enlarge the number of rats reported in the present study. There were four definite differences in the conduct of the experiment: the operation was performed at the age of 1 day; the litters were limited to six rats on the day of operation (the discards are not included in the tables); about 0·3 p.c. of dried baker's yeast was added to the diet, as in the case of the second series; and the rats, although all bred in the laboratory, were of three different strains. In addition to this, an epidemic of lung infections ran through the colony during the first half of the experiment, killing several of the mothers during lactation, and also affecting some of the young rats directly, as autopsy showed. This is the reason for the high number of both operated and control rats which died between the age of 2 and 60 days (Table I). If the rats survived the operation 24 hours their chance of a long life was very nearly the same as that of the controls.

The operation as adapted to animals so small requires more speed than in the older rats, but is otherwise identical. Ether was given with a cone, which was removed before the operation was begun. The period of induction was longer than in the older animals, but no animals were lost because of anæsthesia alone. Sterile technique was attempted but could not always be carried out scrupulously because of the technical difficulties, and wound infections were therefore more common than in the first two series. The operation was modified slightly: the upper half

of the sternum was split and the underlying fascia was divided; the two halves of the sternum were held apart by an essential instrument consisting of a bent "invisible" hairpin grasped in a clamp and pulled towards the rat's abdomen by the assistant; the thymus was then exposed, could be seen clearly, and in all but a few cases was removed completely in one piece. In about one-fourth of the cases a right pneumothorax was produced in pulling out the lower tip of the right lobe, but it was not ordinarily fatal. In a few cases the left auricle was torn in removing the left lobe. The sternum was sutured with silk thread in a quarter-inch curved needle. Most of the operative deaths in the first half of the series resulted from piercing the superior vena cava during this suturing. The skin was closed with a continuous silk suture. Wound infections occurred in eight cases and were the probable cause of death in three of them: the rats died at 6, 20 and 28 days of age, respectively; four cases recovered and the fifth died of another cause. The operative mortality for the first ten litters was 27·8 p.c., but with increasing dexterity it dwindled to 12·7 p.c. for the last ten litters; for the whole series it was 23·6 p.c.

The rats were weaned and the sexes separated at 28 or 29 days, and put on the same régime as Series II with the addition of 0·3 p.c. dried yeast. The data are limited to the age and weight at puberty, and no autopsies were done on surviving animals because they were intended for another experiment which is still in progress. It was felt that any remaining thymus tissue must be in the form of an accessory gland, as the thymus was removed in one piece in nearly every case. The breed was unfortunately not uniform: six litters were of Wistar albino stock, four of hooded stock and the remaining ten of the albino stock used in the previous experiments.

The completeness of the thymectomy.

The thymic region and the entire neck area were examined for gross remnants of thymus tissue in all of the operated animals. In Series I there were three rats with remaining fragments of thymus. In Series II and III no incomplete operations were found. The reason for this may be found in the fact that at operation the wound was never closed until it was felt that the thymus was entirely removed. No gross accessory thymus tissue was found, but it may have been present in microscopic quantities. Previous work of Pappenheimer [1914] and others has shown that this is rather uncommon. The amount of material made the serial sectioning of all the neck and chest organs an impossible under-

taking. If accessory thymus tissue was present it was probably in a small percentage of cases, and if it had any effect on the age of puberty it should have shown in the statistical analysis of the age and weight at puberty. The rats used for mating were autopsied at the age of 1 year: no remaining thymus tissues were found.

Growth and development.

The weight curves, the general development and activity, the age at which the testicles descended (the 3rd week) and the transition from infant to adult hair were the same in the control and operated rats.

Data.

The age and weight at puberty.

The mean age and weight at the opening of the vagina and at the first œstrus are given in Table II and Figs. 1, 2, 3 and 4. The probable

Table II. Age and weight at puberty.

	Series I		Series II		Series III	
	Operated	Control	Operated	Control	Operated	Control
Number of rats	26	26	39	40	21	19
Age of opening of vagina, days	58·8 ± 2·5	58·6 ± 1·9	49·4 ± 0·8	47·8 ± 0·7	47·5 ± 1·4	46·7 ± 1·4
Age at first œstrus, days	62·1 ± 2·4	62·3 ± 2·2	51·7 ± 1·0	52·9 ± 1·0	—	—
Weight at opening of vagina, g.	96·0 ± 1·9	102·0 ± 2·1	93·6 ± 1·6	90·8 ± 1·5	93·3 ± 1·5	92·1 ± 1·5
Weight at first œstrus, g.	—	—	98·3 ± 2·5	98·4 ± 1·8	—	—

error of the mean has been calculated and is also given. The formula used for the calculation was $PE_M = 0{\cdot}675 \sqrt{\Sigma x^2}/N$. The only known difference between Series I and II, in so far as food and other experimental conditions are concerned, is that of season. The diet, care and breed (with the exceptions already noted) were the same. The litters were not limited in number. The greater age at puberty and greater probable error in Series I are therefore probably due to the fact that the rats of this series were born in the winter. Just what factor, whether light, temperature, or unknown change in the food, was responsible is not known. The cages were in a steam-heated, well-lighted room. There was some lowering of temperature at night, but the change was no greater than in the adjacent class-rooms and laboratories. There were some rats of other strains in both experiments. Series I included eight hooded rats, but careful

inspection showed that they were all near the mode of the frequency curves for age and weight at puberty. Series II contained twelve albinos obtained from a different dealer, but they, too, did not differ from the stock rats as to age of puberty when plotted on a frequency curve. The mean weight at puberty is less in the spring-born than in the winter-born animals, but the difference is not so marked as in the case of age, and is

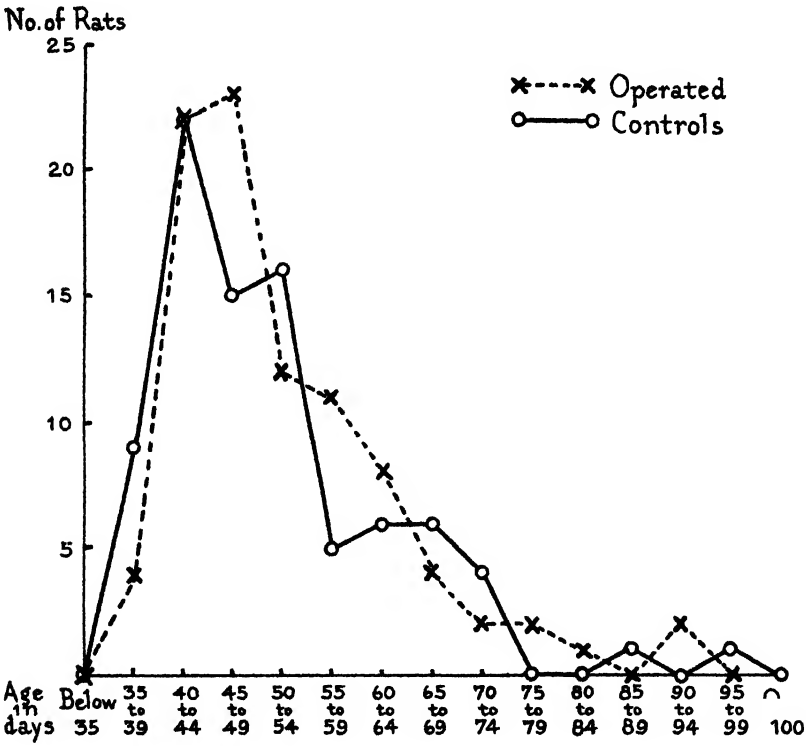


Fig. 1. Frequency curve of the age at puberty of thymectomized and control rats of the three series combined. The criterion of puberty is the opening of the vagina.

not large enough to be significant. It seems obvious from these figures and especially from the frequency curves that the age of puberty can be more closely correlated with weight and general development than with age, and that some factor in the environment of the winter-born rats delayed their growth. The markedly decreased probable error of the mean age of puberty in the spring rats is striking.

The extremes of the age at puberty are 36–95 days, and of weight at puberty 58–134 g., under controlled laboratory conditions. This wide

range indicates that any experiment using age or weight at puberty as a criterion requires large numbers of animals.

The data for Series III are more difficult to interpret. The age and weight at puberty were about the same as in Series II, although various members of Series III were born during the months between December

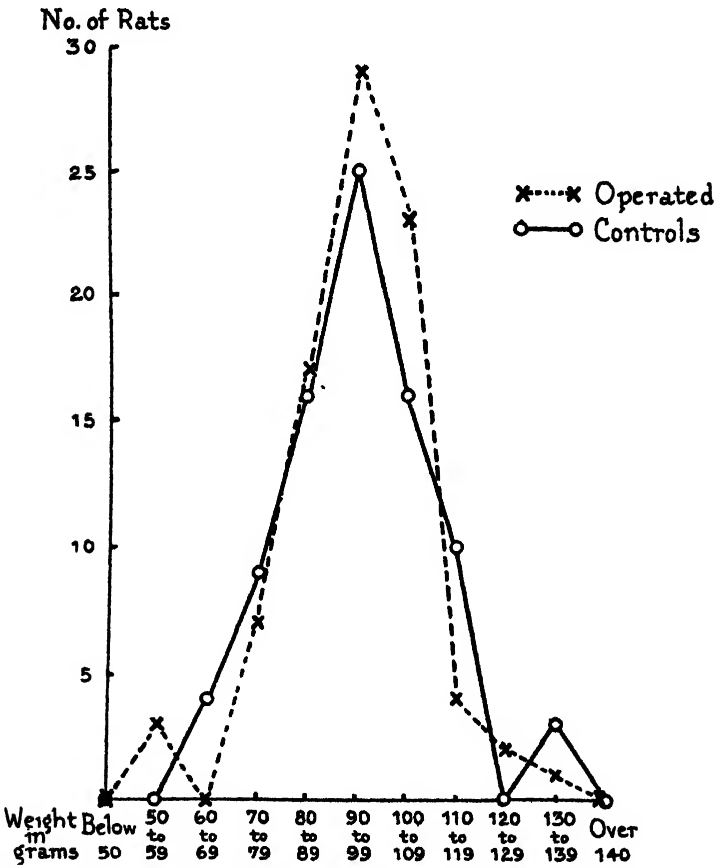


Fig. 2. Frequency curve of the weight at puberty of thymectomized and control rats of the three series combined. The criterion of puberty is the opening of the vagina.

and April. The litters were limited and the diet contained yeast, two factors which make for rapid growth. However, three strains were used, and analysis of the series by strains shows that there is a difference between them in both weight and age at puberty, as shown in Table III. It is not surprising to find that the mean age of puberty of the stock strain of Series III lies between that of the winter-born rats of Series I

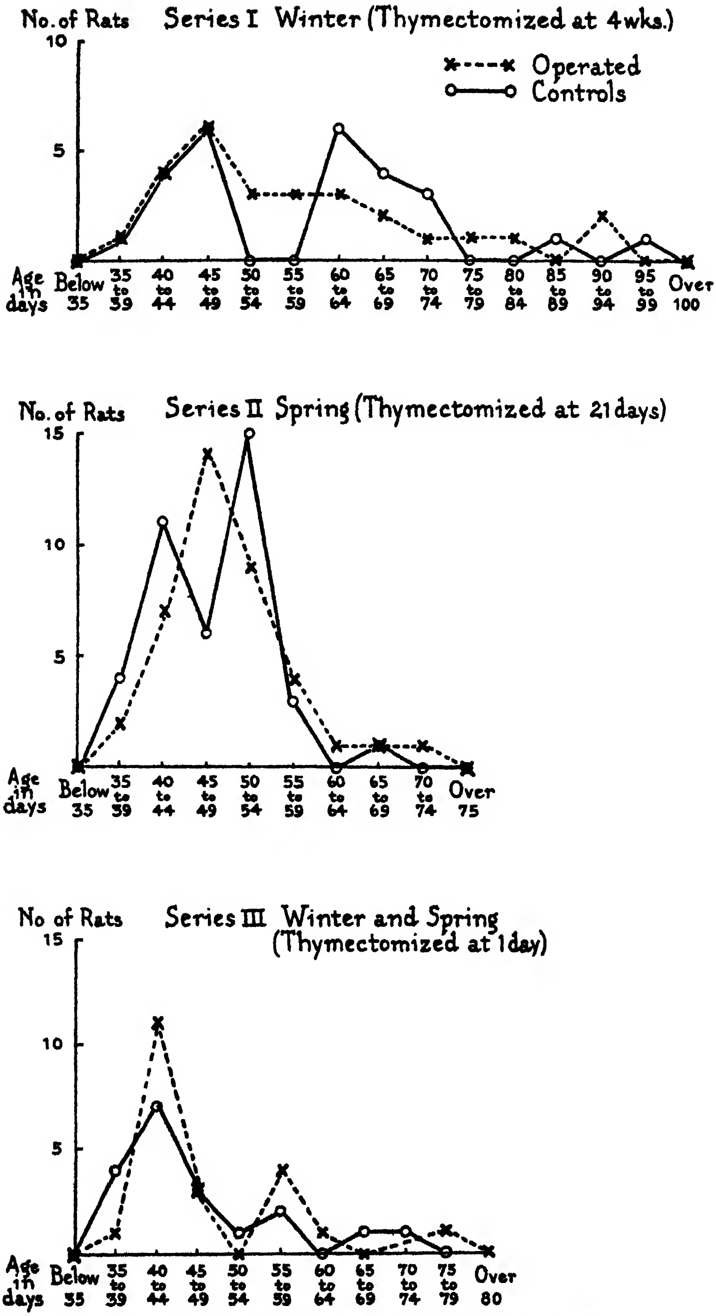
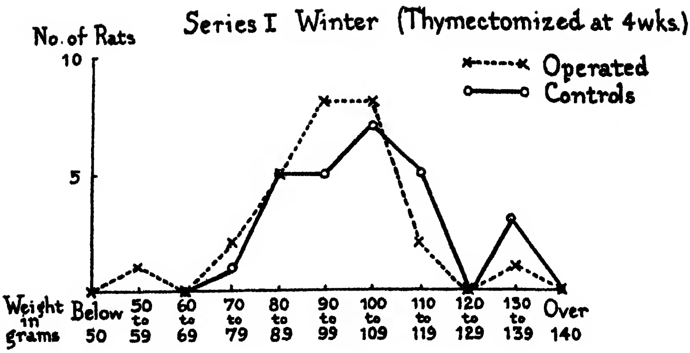
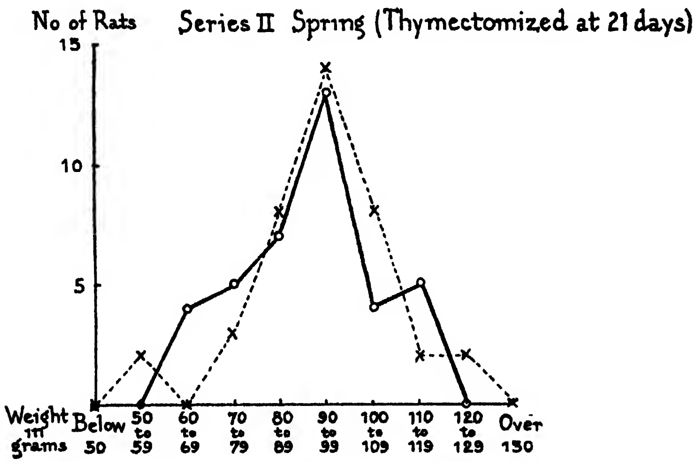


Fig. 3. Frequency curve of the age at puberty for the three series separately. The variation is greater and the mode is later in the winter-born animals.





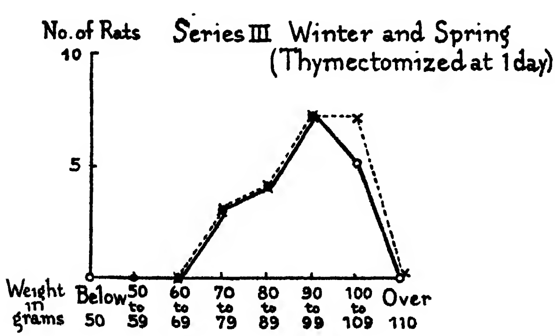


Fig. 4. Frequency curve of the weight at puberty for the three series separately. The curves are smoother than those for the age of puberty. Greater variation and a later mode are found in the winter-born than in the spring-born rats.

Table III. Age and weight at the opening of the vagina in Series III by strains.

Strain	No.	Age (days)	Weight (g.)
McCarrison (stock)	18	50·6±1·7	96·8±1·8
Hooded (G)	11	46·5±1·3	91·0±2·3
Wistar	11	42·0±0·7	87·8±1·6

and the spring-born rats of Series II, which were also of that strain. The hooded rats were slightly below the stock ones in age and weight at puberty. The group of Wistar rats showed a much lower mean age and weight at puberty than the stock albinos. The introduction of this breed lowered the average for Series III. Since litter-mate controls were used in each case this fact does not affect the relative figures for operated as against control animals. The individuals of a litter often reach puberty at about the same age.

The figures show convincingly that the age and weight at puberty are not affected by thymectomy even though the operation be performed at the age of 1 day.

A study of the frequency curve for age at puberty for Series I (Fig. 1) shows that there are two peaks in the control series and one in the operated one. The reason for the second peak was not discovered, since the rats of the odd strain were scattered evenly and inspection of the series with regard to families and ages of weaning revealed no especial grouping to explain it. The symmetry of the weight curve is in striking contrast (Fig. 2).

The interval between the opening of the vagina and the first œstrus.

In Series I the daily vaginal smears of each rat were charted in detail, and from these records a number of observations can be made. The frequency table of the interval between the opening of the vagina and the first œstrus is shown in Table IV. The three animals composing the

Table IV. Interval between the opening of the vagina and the first œstrus in days.

	No. of animals	Interval in days 0	1	2	3	4	5–9	10–14	15–19	20+
Operated	36	11	4	2	10	1	3	4	0	1
Controls	26	11	5	3	0	0	3	2	0	2

group in which this interval was 20 + days were all runts. The average ages of the opening of the vagina for the rats grouped according to the length of this interval are calculated as follows: interval of 0 days, mean age at the opening of the vagina 55·5 days; 1–4 days, 52·75 days; 5–9 days, 70·1 days; 10–14 days, 64·5 days; 20 + days, 56·7 days. This

shows that in the animals in which puberty is delayed, there is likely to be a still further delay in the onset of regular cycles, while a few runts in which the vagina opened at the normal time formed the small group in which this delay is over 20 days.

In only 20 of the 53 rats did regular cycles follow the first œstrus. In the remainder there were one or two or occasionally more œstrous periods at long irregular intervals before the cycles became regular. Once having established a regular rhythm, it was continued with but little variation in the length of the cycles. In only two rats were the intervals still irregular when they were killed at 140 and 141 days.

The actual and relative weight of the thymus, thyroid, adrenals and spleen.

The rats of Series I and II were killed after they had passed through a number of œstrous cycles and were killed at various ages between 70 and 148 days. The adrenals, spleen and thyroid were weighed. In the controls the thymus was also weighed. The relative weight of these organs in terms of mg. per kg. body weight was calculated for each rat. The mean actual and relative weights of each organ were found for the operated and control animals of each series. The probable error of the mean was calculated by the formula given above. The results are shown in Table V. The mean weights of the adrenals and of the thyroid are

TABLE V. The mean weight of the thymus, thyroid, adrenals and spleen.

	Series I		Series II	
	Operated	Control	Operated	Control
Number of rats	23	22	27	24
Body weight at autopsy in g.				
Mean	148	154	141·4	148·7
Range	110–169	120–182	102–177	92–168
Age at autopsy in days				
Mean	110·3	109·7	94·2	94·4
Range	80–148	80–148	70–112	70–112
Thymus:				
Weight in mg.	—	323 ± 22	—	312·58 ± 12·2
G. per kg. body weight	—	1·95 ± 0·08	—	2·17 ± 0·09
Thyroid:				
Weight in mg.	—	—	12·3 ± 0·4	13·1 ± 0·4
G. per kg. body weight	—	—	0·0876 ± 0·0023	0·0993 ± 0·0028
Adrenals:				
Weight in mg.	35·8 ± 1·15	37·6 ± 1·2	35·0 ± 0·7	36·5 ± 0·8
G. per kg. body weight	0·243 ± 0·008	0·251 ± 0·007	0·252 ± 0·004	0·254 ± 0·006
Spleen:				
Weight in mg.	699 ± 24	884 ± 14	538 ± 24	527·2 ± 24
G. per kg. body weight	4·91 ± 0·19	5·85 ± 0·28	3·86 ± 0·15	3·66 ± 0·15

nearly the same in the operated and control rats of both series. The mean weight of the spleen in the winter rats was much greater in the controls than in the operated rats. The spleen of the spring rats had nearly the same mean weight in both operated and control animals, and this weight was much less than either group of winter-born animals. The variability and the probable error was so great that these findings were not really significant. The mean weights of the thymus and adrenals showed no significant difference in the winter and the spring series.

Discussion.

The most obvious criticism of this experiment that presents itself is the absence of proof that the thymectomy was complete. The absence of gross thymus in all but two of the female operated rats is not proof that microscopic fragments are missing. Against this objection can be urged the facts that accessory thymus tissue is uncommon in the rat, and that it is possible to observe the thymus area at operation and to remove the thymus with its capsule intact in the majority of cases. The thymus of a very young rat is a discrete organ, easily separated from adjacent tissues and clearly outlined. It was felt that the chances of leaving portions behind, after experience in the operation, were few.

The evidence offered here disproves the hypothesis that the presence of the thymus is essential for the normal occurrence of sexual maturity. It disproves also the theory that the thymus inhibits the occurrence of puberty. The fact that the thymus is not necessary for normal growth and development already has abundant substantiation. It is possible that the delayed involution of the thymus following castration is not a direct effect of the loss of the gonads; it may be an indirect effect due to the decrease of activity and increase of adiposity, both of which are conditions favourable to the presence of a large thymus. The possible early involution of the thymus following mating and pregnancy (Knipping and Rieder [1924], Jolly and Lieure [1920]) can be interpreted as the result of an increase of activity and of the demand on the nutritional resources of the body, and therefore as the indirect, rather than the direct effect of sexual activity. The abrupt loss in thymus weight during starvation, vitamin-B deficiency [McCarrison, 1921] and acute infections [Hammar, 1926] can also be interpreted by the hypothesis that the thymus is somehow intimately connected with nutrition. Few suggestions as to a specific relation to any food factor have been offered, and there seems to be but little experimental work in the field. Future work will probably be in this direction. It seems futile to search further for a specific

relation between the thymus and reproduction. The function of the thymus remains as mysterious as ever.

The material required for this experiment provides information as to the normal age of puberty in the rat, and also as to the effect of seasonal changes, and of variation in strain. The age of puberty in female rats is given by a number of authors: by Evans at 72 days, by Kinugasa at 60 days. Detailed statistics as to the mean age and weight and the limits of normal variation have not been found. The present data are offered with full realization that the diet, living conditions and size of litters are influential factors. It is possible that the greater age and weight at puberty in our winter series were partly due to the fact that the litters were not limited to six as in later experiments; but because of the operative mortality few litters were larger than that. Since the diet and laboratory conditions were the same, the factor in the different seasons may be temperature or light or something entirely unknown. This problem remains unsolved.

The absence of any difference in the weight of the adrenals, thyroid and thymus in the winter and spring is interesting. Seasonal changes in the adrenals and thymus have been noted by several observers; for example, by Riddle [1923] in the adrenals of pigeons, and by Aimé [1912] in the thymus of turtles. The rats, however, are sexually active and well nourished throughout the year. The turtle and pigeon have definite seasons of reproductive and nutritional activity. The increase in the weight of the spleen during the winter as compared with the spring remains unexplained.

Summary.

Thymectomy experiments have been performed on three series of rats, with a total of 89 operated and 85 control female animals. The operations were done at the age of 2–4 weeks, 21 days and 1 day, respectively, in the three series. The controls were litter mates to the operated animals, and were matched by weight at the age of 1 day. The breed, diet, care and season of birth were controlled, and certain differences in these factors in the three series were noted. The interval between the opening of the vagina and the first œstrus was noted. Autopsies were performed on all the animals of the first two series to ascertain that the thymectomy was complete. In addition, the thyroid, adrenals, spleen and thymus (of the controls) were weighed. The data as to the mean age and weight at puberty are analysed as to season, strain of rats, and presence or absence of the thymus, and are presented with

a calculation of the probable error and with frequency curves. The actual and relative weights of the organs are likewise analysed and charted.

CONCLUSIONS.

A. Concerning the effect of thymectomy.

1. Thymectomy does not affect the age or weight at which female rats reach puberty, when the criterion for the latter is the opening of the vagina. This is true even if the operation be performed at the age of 1 day.

2. Thymectomy does not affect the age at onset of œstrous cycles.

3. Thymectomy does not affect the actual or relative weight of the thyroid, adrenal or spleen when the autopsy is performed at 3–5 months of age.

4. Thymectomy does not affect the growth, appearance or behaviour of young rats, even when the operation is performed at the age of 1 day.

B. Concerning the age and body weight at which the vagina opens.

1. There is a wide variation in normal animals under apparently similar conditions in the age at which they attain puberty. The extremes for age at puberty are 36–95 days. The means for the controls in the three series are 58·6 ± 1·9 (winter), 47·8 ± 0·7 (spring), and 46·7 ± 1·4 days.

2. The weight at which the vagina opens is more nearly uniform as shown by the frequency curves for each series. The age of puberty is therefore more closely dependent on the weight and development than on the age of the animal. The extremes for the controls are 58–139 g. and the means for the controls of the three series are 102·0 ± 2·1, 90·8 ± 1·5 and 92·1 ± 1·5 g. respectively.

3. The age and weight at puberty are less for rats born during the late spring than for those born during the winter, under the same conditions.

4. The age and weight at puberty vary in different strains. Of the three strains used they are lowest in the Wistar rats; next lowest in the hooded rats and greatest in the stock albinos which were used for most of the work.

C. Concerning weights of organs.

1. The thyroid, adrenals and thymus show no difference in the mean, actual or relative weight in winter-born and spring-born animals under laboratory conditions when killed at the age of 3–5 months.

2. The mean weight of the spleen is greater in the winter-born animals, but the variation is so great in our series as to cast doubt on the significance of this finding.

It is a pleasure to express my gratitude to Dr A. M. Pappenheimer for instruction in the technique of his operation, for his good counsel during the experiment and for criticism of the manuscript, and to Mrs Marion Downes and Mrs Helen S. Kennedy for their valuable technical assistance.

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THE EFFECT OF CHICAGO BLUE AND CHLORAZOL BLUE ON THE CLOTTING TIME OF THE BLOOD AND ON OVULATION IN THE RABBIT.

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I. INTRODUCTION.

THE late Dr A. R. Fee had in mind the possibility of performing cross-circulation experiments on the rabbit as a means of investigating certain problems connected with ovulation. Such experiments would involve the use of an anti-coagulant to prevent clotting of the blood for at least 12 hours. The high price of heparine rendered its use on the extensive scale contemplated almost impossible. In considering this problem it was suggested to us by Dr H. P. Gilding that the anti-coagulant properties he and Dr Peyton Rous had observed in Chicago blue 6 B might be utilized. As a preliminary to any cross-circulation experiments it was necessary to investigate the duration of the action of the dye and to ascertain whether it had any effect on ovulation. The present paper is concerned with a brief description of the results of these initial experiments. It was not intended to investigate these properties in detail, but merely to determine whether the dye prolonged the coagulation time sufficiently for our purpose, and to determine the necessary dose. To increase the general usefulness of the work, some additional experiments were carried out on the prevention of coagulation *in vitro*.

II. TECHNIQUE.

Source of dye. The dye employed belongs to the diazo group of the organic dyes. Two samples were used, the first (Chicago blue 6 B) was manufactured by the General Dyestuffs Corporation and purified by Dr Gilding by precipitation and dialysis under toluol [1930]. The second sample (chlorazol sky blue FF) was supplied by the British Drug Houses, Ltd., and was specially purified by them for the purpose of

these experiments. Both are believed to have the same constitution, but in aqueous solution the chlorazol blue was of a lighter tint. Both samples were found to have similar properties.

Administration of the dye. On Dr Gilding's advice, the dye was injected in 8 p.c. aqueous solution, this being isotonic with blood. The solution was freshly made up and boiled before injection. It was found that after standing some days the solution became very toxic, probably owing to bacterial action. Injection was always into an ear vein, the puncture being carefully clipped afterwards. Adult rabbits, 2·5–4·0 kg., were used.

Removal of blood samples. Attempts to obtain the required amount from ear veins sufficiently rapidly were unsuccessful, and it was decided to anæsthetize the animals and remove the blood from one of the large vessels. In the first experiment the animals were lightly anæsthetized with urethane, and then put under ether when the samples were removed. Subsequently, the use of ether was avoided and a heavy dose of urethane given (1·5 g. per kg. followed by 0·3–0·8 g. per kg. as required). In the first experiment the blood was removed from the jugular or the external saphenous by means of a syringe. In later experiments the blood samples were obtained by bleeding directly from the carotid artery into the vessel in which the clotting time was tested. The cut end of the artery was ligatured after each bleeding and the ligatured portion cut off before the removal of the next sample. When animals died during the course of the experiment the last sample was taken directly from the heart immediately after death. Approximately 3 c.c. of blood were removed each time from the injected animals.

Estimation of clotting time. In choosing a method of calculating the clotting time, two difficulties were immediately apparent: (*a*) the prolonged time taken by many of the samples to show any signs of clotting, (*b*) the dark colour of the blood. These points complicated the application of any usual method such as the platinum loop [Gibbs, 1924] or the shot [Dale and Laidlaw, 1912]. Finally, the method used by Dr Pickering in work on hæmophilia was adopted. In the first experiment the blood was drawn into short 1½ in. tubes, kept at room temperature, and the end-point of clotting taken as the time when the blood was sufficiently solid not to flow on tilting the tube at a steep angle. In the second experiment the samples were taken in test-tubes of approximately uniform bore (1 cm.) which had been carefully cleaned, dried and warmed in an incubator at blood temperature (38° C.). The tube was immediately returned to the incubator after the sample was taken. The blood was considered to be

clotted when the tube could be turned upside down without causing displacement. The normal clotting time of the blood of each animal was taken immediately before the injection of the dye.

In vitro experiments. Samples (5 c.c.) were drawn into test-tubes from the carotid of a normal rabbit, 0·5 c.c. of various solutions of the dye being already present. The tubes were left at room temperature, and the criterion of clotting was as in Exp. 2 below.

III. Anti-coagulant effect following intravenous injection.

Exp. 1. The first experiment was performed on three female rabbits injected with 1, 2 and 3 c.c. per kg. of an 8 p.c. solution of Chicago blue 6 B.

The data obtained from this experiment are recorded in Table I. For each sample the time of removal after injection of the dye is given in the first column and the clotting time in the second column.

Table I. Effect of Chicago blue 6 B on the clotting time of blood.

No. of animal	Dose in c.c. per kg.	1st sample hr. min.	1st sample hr. min.	2nd sample hr. min.	2nd sample hr. min.	3rd sample hr. min.	3rd sample hr. min.
CBC 1	1	1 25	0 15+	3 40	0 15	6 37	0 8
CBC 2	2	1 40	—	3 55	—	7 2	—
CBC 3	3	1 55	—	4 0	—	7 5	—

No. of animal	Dose in c.c. per kg.	4th sample hr. min.	4th sample hr. min.	5th sample hr. min.	5th sample hr. min.	6th sample hr. min.	6th sample hr. min.
CBC 1	1	9 11	0 8	19 0	0 8	30 7	0 3+
CBC 2	2	8 11	0 14+	9 30	0 23	18 36	0 13
CBC 3	3	19 5	0 15+	20 30	3 35	•	•

Samples failing to clot under 4 hours are shown by a dash.

Exp. 2. Ten rabbits were employed, pairs being injected with each of the following doses: 1, 1½, 2, 2½ and 3 c.c. 8 p.c. solution of chlorazol sky blue FF per kg. body weight. Half the animals were males and half females.

The results of this experiment are recorded in Table II (arranged as Table I).

Those samples marked with an asterisk were taken from the heart immediately after death. The heavy mortality from 7 hours onwards is to be expected, since the dose of urethane was not much below the lethal point and the animals were suffering from the combined effects of the loss of blood, the operation on the neck and the solution of the dye injected.

TABLE II. Effect of chlorazol sky blue FF on the clotting time of blood.

Number of rabbit	Dose in c.c. per kg. 8 p.c. solution	Equivalent dye per 100 c.c. blood (approx.) g.	Initial coagulation time hr.	min.	1st sample hr.	min.	hr.	min.	2nd sample hr.	min.	hr.	min.	3rd sample hr.	min.	hr.	min.	4th sample hr.	min.	hr.	min.	5th sample hr.	min.	hr.	min.	6th sample hr.	min.	hr.	min.
A	3	0·480	0	3	0	33		—	4	35		—	7	37		—*		.		.		.		.		.		.
B	3	0·480	0	4½	0	31		—	4	33		—	8	20		—*		.		.		.		.		.		.
C	2½	0·400	0	2	0	31		—	4	32		—	8	32		—	12	32		—	16	35	3	47		.		.
D	2½	0·400	0	1½	0	31		—	4	33		—	8	32		—	12	33		—	16	36	3	39		.		.
E	2	0·320	0	5¾	0	31		—	4	34		—	8	33		—	11	25	1	1*		.		.		.		.
F	2	0·320	0	5½	0	31		—	4	34		—	8	33		—	12	32		—	14	35	2	7*		.		.
G	1½	0·240	0	3	0	30		—	4	32	0	26	8	32	0	10	12	32	0	15	16	31	0	4		.		.
H	1½	0·240	0	4½	0	31		—	4	32	0	24	8	32	0	10	12	33	0	6	15	41	0	6*		.		.
I	1	0·160	0	7	0	31	0	19	4	33	0	7	8	32	0	4	12	34	0	2	16	31	0	2	19	59	0	1½
J	1	0·160	0	4½	0	32	0	58	4	34	0	14	7	9	0	17*		.		.		.		.		.		.

TABLE III.

Dose in c.c. per kg.	Hours after injection of dye: 1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th	17th	18th	19th	20th	21st
1	++	+	.	+	++	.	+	+	+	+	.	.	+	.	.	.	+	.	+	+	+
1½	00	.	.	.	++	.	.	.	++	.	.	.	++	.	.	+	+	.	.	.	.
2	00	0	.	0	00	.	.	0	00+	+	.	+	0	.	+	.	.	.	+	.	.
2½	00	.	.	.	00	.	.	.	00	.	.	.	00	.	.	.	++	.	.	.	.
3	00	0	.	0	00	.	.	00	0	.	.	.	.	.	.	.	.	.	.	+	+

The results show clearly that the dye prolongs the coagulation time of blood beyond measurable limits for some time after injection. Both the intensity and the duration of the anti-coagulant effect are directly proportional to the dose given. This conclusion is shown more clearly if the results are tabulated as in Table III, in which the results of Exps. 1 and 2 are combined. The plus signs indicate that the samples clotted to standard under 4 hours after removal and the zero signs that they failed to do so.

The results recorded in Tables I and II appear to show that the

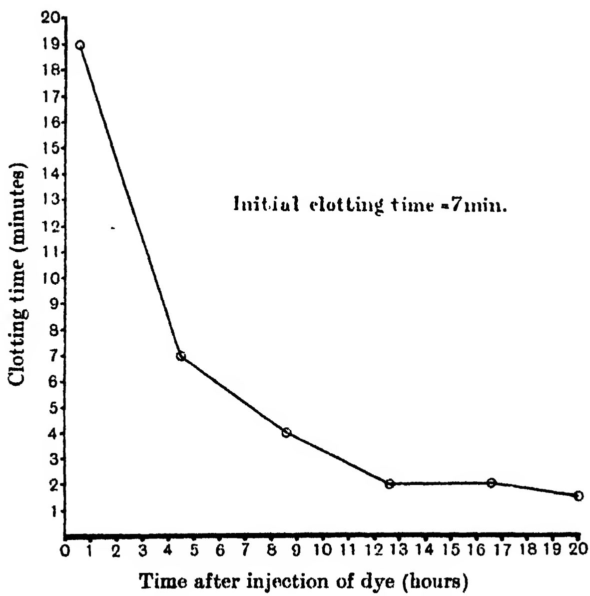

Fig. 1. Anti-coagulant effect of chlorazol blue given intravenously (Animal 1, Exp. 2).

clotting time of the blood reaches a maximum immediately or very soon after the injection of the dye. It then falls off continuously, at first rapidly and then increasingly slowly.

The general type of curve obtained by plotting the clotting time against the time after injection is represented in Fig. 1, in which the data for Animal 1 are plotted. The data for the other animals, although not so complete, support the assumption that the clotting time after injection falls from an initial maximum in a similar manner. The example figured is noteworthy, in that the clotting time after injection falls below the normal clotting time as determined before the dye was injected.

IV. *In vitro* experiments.

The highly effective intravenous dose of 2·5 c.c. of 8 p.c. solution per kg. represents about 0·4 g. per 100 c.c. blood before diffusion begins. It was anticipated that much less would be required *in vitro*, and the

Table IV. Effect of adding chlorazol blue to blood *in vitro*.

Sample No.	Solution of dye of which 0·5 c.c. added to 5 c.c. blood (p.c.)	Dye in g. per 100 c.c. blood	Clotting time hr.	min.
0	—	—	0	3½
4	0·1	0·010	0	4
3	0·5	0·050	0	5½
2	1·0	0·100	0	7½
13	1·0	0·100	0	8½
12	1·25	0·125	0	28½
9	1·5	0·150	1	44
11	1·75	0·175	Failed to clot in 18 hours	
1	2·0	0·200	,,	,,
8	2·5	0·250	,,	,,
7	3·0	0·300	,,	,,
6	3·5	0·350	,,	,,

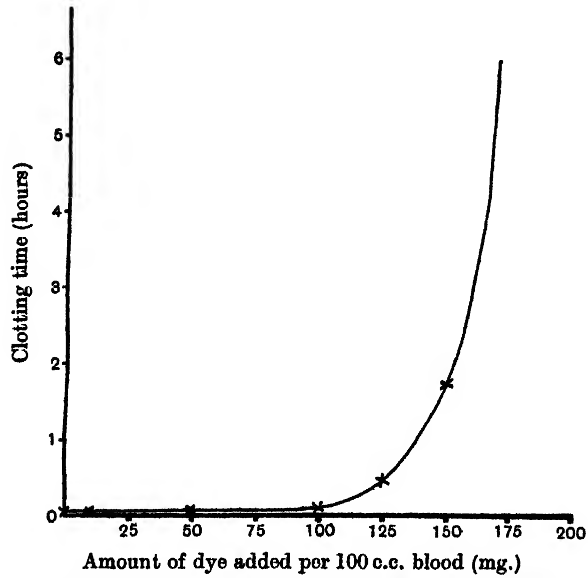

Fig. 2. Anti-coagulant effect of chlorazol blue *in vitro*.

amounts added to the samples ranged from the equivalent of 0·010 g. up to the equivalent of 0·350 g. per 100 c.c. of blood (details in Table IV). To keep constant the amount of fluid (0·5 c.c.) added to the blood sample, solutions were made up as shown in Table IV. From this

table it will be seen that 0·150 g. of dye per 100 c.c. blood inhibits clotting for nearly 2 hours, while 0·175 g. of dye per 100 c.c. blood prevents clotting indefinitely. Clotting time plotted against amount of dye added (Fig. 2) gives a type of curve which suggests that some critical reaction begins as the amount of dye added rises above 0·100 g. per 100 c.c. of blood.

The smallest intravenous dose given represented about 0·160 g. per 100 c.c. blood, an amount which 30 min. after injection prevented clotting for an average time of nearly 40 min. Allowing for the 30 min. interval, it is evident that the intravenous results are strictly comparable with those obtained *in vitro* and that the efficiency of the dye in the two conditions is similar.

V. Effect on ovulation.

In some early experiments in which the dye was injected after copulation, two rabbits failed to ovulate. Further work, however, did not reveal any constant disturbance of ovulation. Details of the eleven animals used are given in Table IV.

Table IV. Effect of Chicago blue 6 B on ovulation.

Number of animal	Dose in c.c. per kg. 8 p.c. solution	Time of injection *post coitum* min.	Number of follicles ovulating
SOR 1	2·5	20	0 + 0
SOR 2	2·5	46	0 + 0
SOR 4	2·5	40	2 + 2
SOR 5	1·0	35	2 + 3
SOR 6	2·5	45	3 + 4
SOR 7	2·5	40	0 + 0
SOR 8	2·5	7	3 + 4
SOR 9	2·5	4	0 + 0
SOR 10	2·5	4	1 + 0
SOR 11	2·5	20	1 + 0
CBC 4	2·5	40	5 + 1

The dose was large in all animals except SOR 5, and it was administered at various times during the first hour *post coitum*. Of the four animals which failed to ovulate, one (SOR 9) had definitely immature follicles, and one (SOR 7) exhibited pre-ovulation changes before death at 13 hours *post coitum*. In view of the fact that about 20 p.c. of normal rabbits fail to ovulate after copulation, these results show conclusively that the dye in the doses given does not inhibit ovulation.

V. Summary.

1. Chicago blue 6 B (chlorazol sky blue FF) given intravenously has a marked anti-coagulant effect on the blood of rabbits. 2·5 c.c. per kg. of

an 8 p.c. solution prolongs the clotting time to over $3\frac{1}{2}$ hours in samples taken 16 hours after injection. Samples taken earlier than this fail to clot in a significant time. After injection of 1 c.c. per kg. the clotting time returns to normal in about 4 hours.

2. Similar doses failed to show any adverse effect on ovulation.

3. The dye is therefore highly suitable for work on ovulation requiring the use of an anti-coagulant.

4. *In vitro*, 175 mg. of the dye per 100 c.c. of blood inhibits clotting indefinitely.

We are much indebted to Dr Gilding for suggesting to us the use of the dye and for providing us with specially purified material. We are also indebted to Dr F. H. Carr of the British Drug Houses, Ltd., for having the second sample specially purified; to Dr J. W. Pickering for valuable advice on the technique of measuring the coagulation time of the blood, etc.; and to Miss M. Hill who assisted with some of the initial experiments.

The expenses of the research were defrayed by a Grant from the Government Grants Committee of the Royal Society to one of us (F. W. R. B.).

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THE BODY TEMPERATURE IN RATS ON NORMAL AND DEFICIENT DIETS.

Preliminary Report.

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PREVIOUSLY, I have emphasized the importance of clinical examinations of the animals in nutrition experiments [Gudjonsson, 1930*a*]. But the investigations reported in this paper were really undertaken on account of my observations on the periocular reaction [Gudjonsson, 1930*b*]. I had interpreted the periocular reaction as a sort of focal reaction or demarcation process, appearing when A-avitaminotic rats are supplied with a considerable addition of vitamin A. It was then natural to imagine that this focal reaction would be accompanied by a rise of body temperature, a feature that is well known from clinical observation in inflammatory reactions. On autopsy, however, the A-avitaminotic animals show but a very slight reaction around the numerous abscesses usually encountered—*e.g.* at the base of the tongue, in the cervical glands, urogenital organs, etc. These severe infections often take a chronic course without any particular reaction in the surrounding structures.

So my working hypothesis was as follows: on account of the vitamin A deficiency, the organism is incapable of reacting normally to these focal infections. And an animal supplied with an adequate addition of vitamin A will again be able to react with the acute processes of inflammation and resulting fever. But the results of my experiments have not corroborated this working hypothesis. The results are about the opposite of what I had expected. Still, I did find something—and it may be that further investigation along this line will throw more light on the rather obscure patho-physiology of A-avitaminosis.

Pembrey [1895] has examined the rectal temperature of adult rats with a mercury thermometer, and he finds the normal temperature to be 37·5° C. MacLeod [1907] has also used a mercury thermometer to

measure the rectal temperature in adult rats, and in his experiments it varies between 37·5 and 38·5° C., with an average of 37·9° C. He further finds that if the temperature of the air rises above 37·5° C., the body temperature of the rats rises rather rapidly, and the animals die with hyperpyrexia. Congdon [1912], who also employs a mercury thermometer, finds 37·9° C. as the normal rectal temperature in young rats; in adult rats, living at an environmental temperature of 16° C., he finds a body temperature of 36·2° C., whereas he finds a body temperature of 37·2° C. in adult rats living at an environmental temperature of 33° C. In new-born rats the body temperature is subject to great variation, dependent upon the environmental temperature. When the rat is 10 days old its body temperature is more stable, though it is never altogether well regulated [Donaldson, 1924]. The body temperature is said to be higher in female rats than in males, and it falls a little in the evening [Bierens de Haan, 1922*a*, *b*]. Graham and Hutchinson, [1914], who employ a thermoelectrical measuring method, find that the body temperature in rats varies greatly with variations in the environmental temperature. Thus, several investigators have found that the normal body temperature of the rat is about 37·5–37·8° C., and that it is rather unstable, with a tendency to poikilothermia.

According to the aforementioned hypothetical view as to the reaction of A-avitaminotic rats when supplied with vitamin A, I have made two series of experiments that are partly parallel.

Exp. 1. Ten rats of the same litter, 30 days old and weighing *ca.* 50 g., are first placed on the usual vitamin A-free diet. Each rat is kept in its separate wire cage, and they all stay in a room with a constant temperature of *ca.* 22° C. Every day, at the same time (about 2 P.M.), the rats are brought into an examination room where the temperature is also about 20–22° C. After staying in this room about half an hour, the rectal temperature is taken with a thermoelectrical probe, measuring *ca.* 1·5 mm. in diameter. Before and after this procedure, the accuracy of the thermoelectrical apparatus is checked by comparison with a standardized mercury thermometer; and, of course, the temperatures obtained in the rats are corrected accordingly if any correction is to be made. After the rats have been on this diet for about 3 weeks, they begin to show clinical signs of A-avitaminosis: they stop gaining in weight, and the first symptoms of xerophthalmia make their appearance. The rats are kept a couple of weeks more on the vitamin A-free diet, so as to ensure that they all have focal infections. They are then divided into two groups. The first group of 5 rats is now supplied with a daily addition

of shark-liver oil—50 mg. daily per rat, which is ten times the optimal dose of vitamin A. The other group is kept on the vitamin A-free diet.

Two rats of the first group, which had an addition of vitamin A, died 1–2 days after they were supplied with this addition. Of the remaining 3 rats, 1 lived 4 weeks on this diet with addition of shark-liver oil, and 2 lived throughout the experimental period, *i.e.* 8 weeks. These 3 rats recovered from the symptoms of A-avitaminosis, and they gained

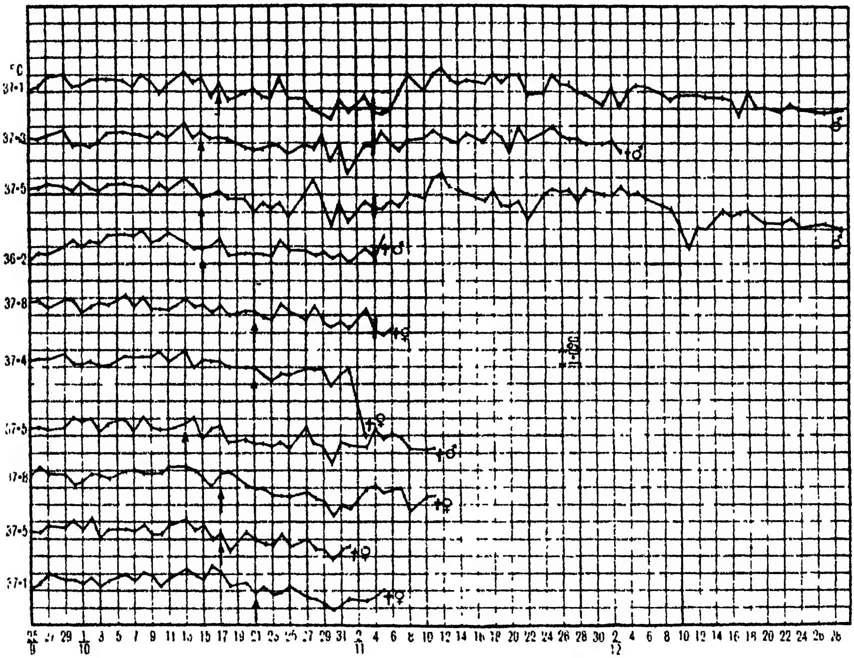

Fig. 1. Curves of rectal temperature in rats on vitamin A-free diet with and without addition of vitamin A. The arrow indicates the time of the appearance of xerophthalmia. The vertical line shows the time when the rats are first supplied with shark-liver oil.

considerably in weight, though hardly to a normal degree—they had been too ill before they were supplied with vitamin A. In fact, the autopsy revealed signs of severe pathological changes, urinary calculi and nephritis.

During the first 3 weeks, all the rats show a fairly constant rectal temperature, averaging 37·5° C., ranging from 36·2 to 38·2° C., as shown in Fig. 1. About the time when the clinical symptoms appear, or a little before, the rectal temperature begins to be less stable and shows a

tendency to fall. The next 2 weeks the rectal temperature averages 36·6° C.—*i.e.* 1° below the normal—ranging from 35·2 to 37·9° C. After the rats had been getting a daily addition of vitamin A, they show some remissions of the rectal temperature with a tendency to a higher level. Still, the rectal temperature does not reach the level registered in the same rats before the A-avitaminosis, but stays about ½° lower—averaging

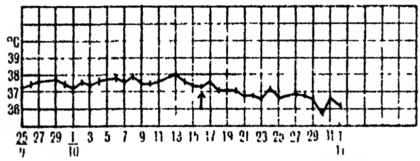

Fig. 2. Average curve of rectal temperature in 5 rats on vitamin A-free diet. The arrow indicates the time of the appearance of xerophthalmia.

36·9° C. In this period the highest measured temperature is 38·3°, the lowest 34·0° C.

Fig. 2 gives the average curve of rectal temperature in the 5 rats of group 2 which were kept on a vitamin A-free diet throughout the experimental period. It illustrates very clearly the fall of the body temperature of these rats in the period of A-avitaminosis.

Fig. 3 shows the average curve of rectal temperature in A-avitaminotic rats that are having their after-period prolonged by addition

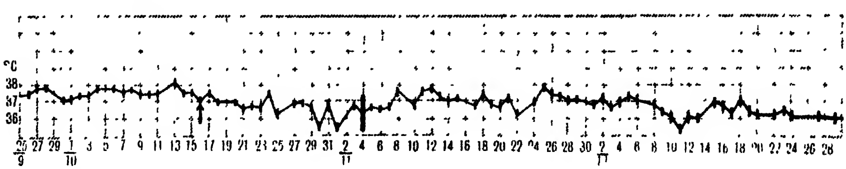

Fig. 3. Average curve of rectal temperature in 3 rats on vitamin A-free diet plus an addition of shark-liver oil in the after-period. The arrow indicates the time of the appearance of xerophthalmia. The vertical line shows the time when the rats are first supplied with shark-liver oil.

of shark-liver oil to the vitamin A-free diet. The curve illustrates the fall of the temperature during the period of avitaminosis and the subsequent irregular rise of the temperature which does not reach the normal level.

In this series of experiments, then, one finds the peculiar outcome that when the animals begin to suffer from A-avitaminosis and are ill with xerophthalmia and other infections, their body temperature falls on an average *ca.* 1° C. below the normal. If these animals are now supplied with vitamin A so that they apparently recover and their

growth is restored, their body temperature rises to some extent but not up to the normal level. I had expected that the animals would have fever in the beginning of this period of improvement. But this was absolutely not the case. In human individuals with such infections as are found in the rat during the A-avitaminotic period, one would find a marked elevation of the temperature.

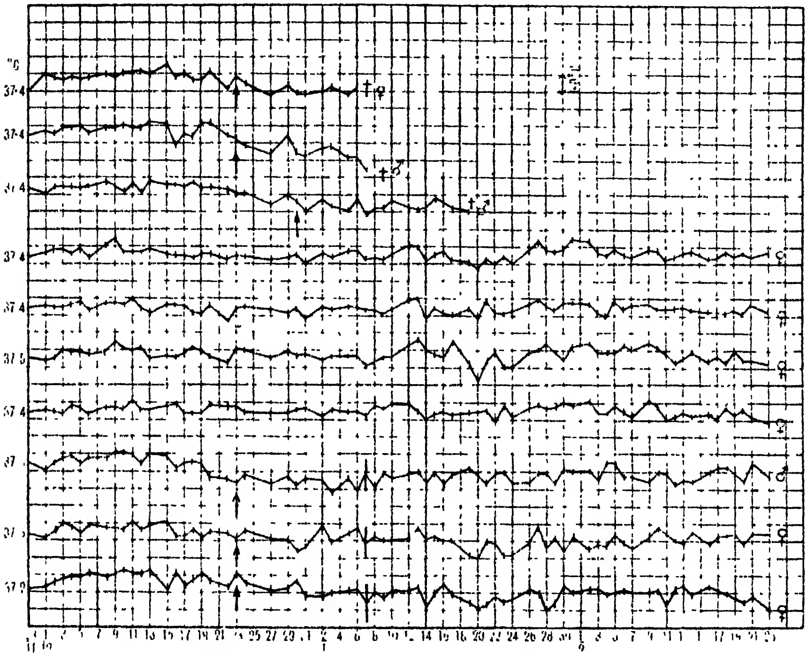

Fig. 4. Curves of rectal temperature in rats on normal and vitamin A-free diets. The upper three come from rats on vitamin A-free diet, the middle four from rats on normal diet, and the lower three from rats on vitamin A-free diet plus an addition of shark-liver oil in the after-period. The arrows indicate the time of the appearance of xerophthalmia. The vertical line shows the time when the rats are first supplied with shark-liver oil.

To corroborate this unexpected result, the following experiments were performed.

Exp. 2. Ten rats of the same litter, 30 days old, were divided into two groups. Four rats were placed on the normal rat diet employed in this laboratory—6 were placed on the usual vitamin A-free diet. All the animals lived under the same experimental conditions as described above, and their rectal temperature was measured in the same way as above. The experiment lasted 88 days.

Fig. 4 gives the temperature curves in this experiment. The results are quite in keeping with those obtained in Exp. 1.

The average temperature of the normal rats lies midway between 37 and 38° C. This is also evident from Fig. 5, which shows a curve for all the rats in the period before any of the rats showed signs of A-avitaminosis, and before the temperature began to fall. This is further illustrated in Fig. 6, which shows the average temperature of the 4 rats on normal diet throughout the experimental period. Here the values are also between 37 and 38° C. So the normal temperature of the rat as obtained in these experiments agrees with that reported by other investigators as mentioned in the beginning of this paper.

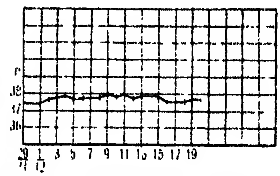

Fig. 5. Average curve of rectal temperature in 10 rats, covering a period of 20 days, during which 4 have normal diet while 6 have vitamin A-free diet.

Of the 6 rats that were placed on vitamin A-free diet, 3 were kept on this diet till they died. As in the preceding experiment, the temperature

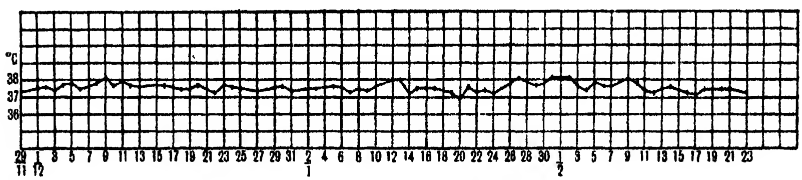

Fig. 6. Average curve of rectal temperature in 4 rats on normal diet.

of these rats began to fall some days before they showed signs of xerophthalmia. The temperature continued to fall, and just before death it was

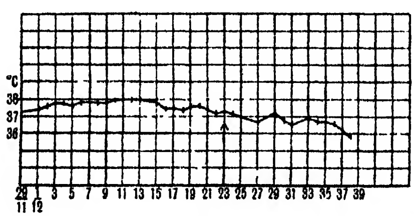

Fig. 7. Average curve of rectal temperature in 3 rats on vitamin A-free diet. The arrow indicates the time of the appearance of xerophthalmia.

1·5° C. below the average temperature registered by the same rats in the period prior to the A-avitaminosis—see Figs. 7 and 4. The other 3 rats of this group on vitamin A-free diet had in the after-period an addition

of shark-liver oil (about ten times the optimal dose). In this experiment the shark-liver oil was given earlier in the after-period than in Exp. 1. Apparently, all 3 rats recovered. All signs of xerophthalmia disappeared, and the rats were gaining in weight. The rectal temperature of these rats showed the same peculiarity as that of the corresponding rats in Exp. 1: it rose, but it never quite reached the normal level nor its level in the same rats during the period prior to the A-avitaminosis, the average temperature of the after-period being about 0·6° C. lower than that of the fore-period—see Figs. 4 and 8.

This experiment shows, then, that in A-avitaminosis the temperature of the rat falls to a level 1–2° C. below the normal. Still, as mentioned before, in this period the rats are suffering from extensive infections. When such rats are supplied with an adequate amount of vitamin A,

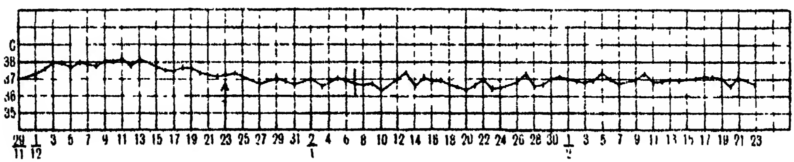

Fig. 8. Average curve of rectal temperature in 3 rats on vitamin A-free diet plus an addition of shark-liver oil in the after-period. The arrow indicates the time of the appearance of xerophthalmia. The vertical line shows the time when the rats are first supplied with shark-liver oil.

they get no fever, but their temperature curve shows a slow and gradual but incomplete rise. The striking peculiarity is the fact that the temperature does not quite reach the normal level. It may be that it might have reached the normal level if the experimental period had been extended further, but it is not very likely.

These experiments further confirm what has been found before, that the body temperature of the rat is rather unstable. I shall not advance any interpretation of these results. But they suggest that A-avitaminosis lowers or alters the metabolism; or perhaps it affects the thermoregulating apparatus of the animals in some way or other.

Summary.

1. In two series of experiments the body temperature in rats on normal and vitamin A-free diets was measured.

2. The body temperature is rather unstable, but normally it lies midway between 37 and 38° C.

3. In A-avitaminosis the temperature falls, and in the last part of the A-avitaminotic period it lies about 1·5° C. below the normal.

4. On addition of large amounts of vitamin A to the vitamin A-free diet, the rats under this treatment show a rise of temperature, though this does not reach the normal level—not in 8–10 weeks, at any rate—notwithstanding the fact that in all other respects these animals recover from the A-avitaminosis apparently completely.

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THE VAGUS INFLUENCES GIVING RISE TO THE PHENOMENA ACCOMPANYING EXPANSION AND COLLAPSE OF THE LUNGS.

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Introduction.

It is probable that the generally accepted hypothesis regarding the regulation of the breathing through nervous channels is still that owing to the reflex effect of volume changes in the lungs each phase of respiration automatically inhibits itself and excites the succeeding phase. Thus inspiration is cut short at a certain depth by stimuli arising from the expansion of the lungs, the expansion exciting an active expiratory reaction, while collapse at a certain point inhibits expiration and excites an inspiratory discharge from the centre. This is the hypothesis first enunciated by Hering and Breuer [1868] and described by them as "the auto-regulation of respiration through the vagus." This hypothesis was based on the interpretation of the effects produced (*a*) by closure of the tracheal outlet at the height of inspiration and expiration respectively, and (*b*) by inflation of the lungs by air under pressure or deflation by suction or puncture of the pleura.

Haldane and Mavrogordato [1916] have repeated the experiment in normal human subjects using the first method, Christiansen and Haldane [1914] using the second method, and believe that their results confirm on the whole the conclusions of Hering and Breuer, although they also conclude that the points at which inspiration or expiration is inhibited depend on the chemical stimulus to the respiratory centre.

Gad [1880], who followed Rosenthal [1862] in believing that change in the oxygen content of the blood was the chemical stimulus to the centre, in addition to observations of the effects of inflation and deflation drew his conclusions largely from the changes he found to occur in the form of the respiratory cycle following section of the vagi. He did not find that section necessarily led to any great increase in depth or decrease

in rate of breathing. He describes the characteristic effect as being a great prolongation (spasm) of the inspiratory phase at the expense of the resting phase of expiration, an inversion of the normal cycle, there being thus a wasteful expenditure of energy in producing the required ventilation. He agreed with Hering and Breuer in thinking that expansion of the lungs in inspiration terminated that act by reflex inhibition. He differed from them in denying that expansion excited an active expiratory act or that collapse (in expiration) excited the inspiratory discharge. He believed, however, that the inhibitory influence on the centre set up by inspiration does not end with inspiration but remains as an after-effect which gradually dies away, the following inspiratory discharge being determined by the moment at which the chemical excitability of the centre overbalances the diminishing inhibitory effect. Gad's view thus implies the existence of only one type of vagus termination in the lungs which is excited by expansion.

Head [1889] confirmed Hering and Breuer's results as regards the inhibitory effect of inflation on inspiration and the inspiratory response to deflation or puncture of the pleura. He did not find any active expiratory response to expansion of the lungs. He agreed with Gad in thinking that the inhibitory influence of expansion during inspiration has an after-effect in the same direction to which he ascribes the expiratory pause. With that author he regarded expiration as a passive state.

The increased inspiratory tonus or spasm arising from collapse, and particularly the summation effect following repeated negative ventilation, which Head observed, showed in his opinion that collapse of the lungs raised the inspiratory activity of the centre and had an after-effect analogous, but in the opposite direction, to that resulting from expansion.

His observations thus supported the Hering-Breuer hypothesis in supposing that the vagus transmitted two opposite influences to the centre, the one terminating, the other initiating inspiration, two sets of vagus terminations in the lungs being implied, excited respectively by expansion and collapse, but opposed that hypothesis in denying the existence of any reflex stimulus to expiration.

The results obtained by some more recent observers will be referred to in the text.

It is proposed in this paper to consider the function of the nervous influences arising in the lungs, which affect the breathing, by comparing the results of artificial changes in the pulmonary volume and the changes which occur in the respiratory cycle following section of the vagi.

METHOD.

Previous observers have without exception employed methods of inflation or deflation depending on the production of positive or negative air pressures within the lungs and air passages or the sudden closure of the air outlet at the end of either the inspiratory or expiratory phase. The methods of recording have been various, either by means of a manometer connected to the air passage, by registration of the movements of the thoracic walls, different modifications of Gad's pneumograph [1879] or by Head's method using the diaphragmatic slip.

With the exception of Gad's method none of the above gives a correct representation of the volume changes occurring during the whole respiratory cycle such as is required if exact observations are to be made on the time relations or reflex modifications of the various phases of respiration.

The authors have used a method in which no change in the intrapulmonary pressure takes place, inflation and deflation being produced respectively by a negative or positive air pressure acting on the external body surface of the animal. The method of recording was on the same principle as that devised by Gad, a light bellows being used to record the volume changes in the expired and inspired air. The method, which imitates the natural means by which expansion and collapse of the lungs are brought about, is thought to eliminate any abnormal reflex effects which might be caused for example by pressure changes within the lungs.

The apparatus employed throughout the observations recorded below has been described elsewhere [Wilson and Hammouda, 1928].

Briefly the apparatus consists of a chamber or box sufficiently large to contain a 7 kg. dog. The chamber has an airtight cover which is closed after the introduction of the animal. A tube connected to a tracheal cannula passes through the cover to a large air reservoir, to which is attached a bellows recorder of about 700 c.c. capacity.

The volume and rate of respiration are recorded in this way, the animal breathing in and out of the reservoir. The dead space of the tubing between the trachea and the reservoir is about 20 c.c.

Expansion of the lungs is produced by withdrawing air from the chamber, so causing a negative pressure around the animal; deflation by the opposite procedure. The pressure within the air space is recorded by a mercury manometer connected to the interior of the box.

Dogs weighing from 3½ to 7 kg. have been used, anæsthetized

(Morphine 5 mg. per kg. C. and E. Chloralose 0·6 g. per kg.) or decerebrated (in front of the anterior colliculi). The corneal reflex was active in all the animals from which records have been preserved.

The effects of volume changes and of vagotomy respectively were the same in chloralosed and decerebrate animals.

Section I.

The form of the cycle and the time relations of the phases of respiration.

Before proceeding to describe the effects of volume changes in the lungs it is necessary, for a correct appreciation of the meaning of those effects, to consider briefly the form of the respiratory cycle under normal conditions[1] and after section of the vagi.

Fig. 1. The form of the respiratory cycle in the dog. Dog *F*, wt. 7 kg. Morphine 25 mg. Chloralose 0·4 g. Temp. 38·6° C. Rate of breathing (average) 10 per min. Depth 150 c.c. Cycle *a*, *b*, *c*, *d*. Total duration 6·6 sec. Phases *a*, *b*, inspiration 0·9 sec.; *b*, *c*, expiration 1st phase 0·7 sec., *c*, *d*, 2nd phase (pause) 5·0 sec. Bellows recorder. Kymograph medium fast rate. To be read from left to right. Inspiration down, expiration up.

As stated above (p. 81) Gad based his views regarding the function of the vagus in respiration mainly on the form of the respiratory cycle before and after vagotomy. His description [1879] of the normal cycle is the same as that illustrated above (Fig. 1).

Head [1889] (above, p. 82), "using any form of aero-plethysmograph," gives a similar account, but describes the third phase as occurring occasionally as follows: "the pause appears to give rise to a slow but ever increasing contraction of the expiratory muscles"; this active expiratory phase will be referred to later as often accompanying inflation.

The above figure shows the normal form of the cycle in the decerebrate or chloralosed dog.

[1] See Appendix at end of this Section, p. 91.

It will be seen that the cycle consists of three well-defined phases. The inspiratory phase extends from *a* to *b*, the 1st phase of expiration from *b* to *c* and the 2nd phase from *c* to *d*. The writers believe that the rapid 1st phase of expiration is mainly due to elastic recoil, but accompanying this and largely responsible for the 2nd phase must be the restoration of tonus in the internal intercostals inhibited (reciprocally) during the inspiratory phase.

The time relations were in the example shown in the figure as follows: duration of inspiration 0·9 sec.; expiration 1st phase 0·6 sec.; 2nd phase 4·5 sec. The rate of breathing was 10 per min. (average figures).

The duration of the phases varies in different animals, but, with the exception of the pause (2nd phase of expiration) *c* to *d*, is very slightly affected by changes in the rate or depth of breathing from whatever cause except a rise of body temperature (heat polypnœa).

In seven animals in which the rate was from 8 to 42 per min. and depth from 60 to 330 c.c., the duration of the inspiratory phase varied from 0·6 to 1 sec. (it is sometimes longer, *vide* Fig. 2), but was nearly constant in individual cases with varying rate and depth. The average duration was 0·82 sec.

The 1st phase of expiration had an average duration of 0·5 sec., varying from 0·3 to 0·7 sec. in the different animals. With increasing rate and depth due to asphyxial conditions the duration of this phase is slightly increased, a result which would be expected if in this phase the discharge of air is mainly due to the passive elastic recoil of the thoracic walls and lungs.

The 2nd phase of expiration (pause) varied in duration in animals with a different rate of breathing from 1·3 to 6 sec. in the absence of any anoxæmic or asphyxial condition. In asphyxial conditions it may be reduced to 0·1 sec.

It will be seen, therefore, that in dogs the rate of respiration depends almost entirely, in both normal and asphyxial states, on the duration of the pause indicated in the figure as the 2nd phase of expiration.

The record of the pause in the case illustrated is horizontal, showing the undulations due to the cardiac movements, but no change whatever in the volume of the lungs. In some animals the line rises slightly, but in no case (apart from inflation) has Head's 3rd stage of expiration been seen.

The salient fact in regard to this phase is the absence of any change in the lung volume immediately preceding inspiration, the impulse to which cannot therefore be ascribed to a reflex excitation arising peri-

pherally from collapse of the lungs. It is evident that some indication of the effect of collapse should be visible if that were in reality the stimulus to inspiration[1].

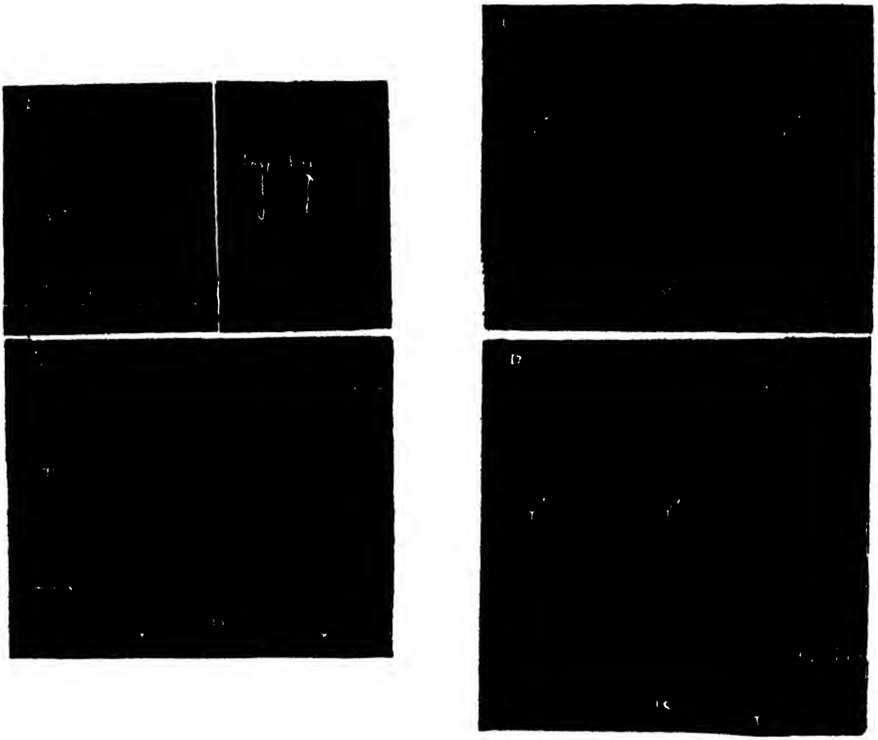


Fig. 2. The respiratory cycle after vagotomy. Dog, wt. 5½ kg. Morphine 0·3 c.c. 10 p.c. solution. Chloralose, 0·4 g. Bellows recorder. Inspiration down, expiration up. To be read from right to left.

Record	Rate per min.	Depth in c.c. of breathing	Duration of phases Insp.	Exp. 1	Exp. 2
			sec.	sec.	sec.
A. Normal before section	12	105	1·5	0·9	2·7
B. Immediately after section	5	400	2·7	1·6	7·0
C. 30 min. later	4·5	205	3·0	0·9	8·6
D. Later, effect of rebreathing	8	550	2·9	1·6	2·1

The arrow marked *T* on each curve indicates a point 1·5 sec. after the beginning of inspiration. The expansion reached in the four cases at this point was in A 105, B 115 C 105 and D 210 c.c.

[1] Taylor and Taylor's observations [1931] on dogs with increasing HCN poisoning also show that in the absence of any stimulus arising from either active or passive expiratory collapse of the lungs rhythmical inspiratory discharges can be initiated by the centre.

This finds further support in the fact that the form of the curve and the relative duration of the phases after vagotomy are unchanged. This is illustrated in Fig. 2, in which A is a record taken immediately before section, B just after, C half an hour later, and D still later, showing the effect of rebreathing.

From the records A, B, C, and D in the figure and the measurements tabulated it is evident that the form of the cycle and the relative time relations of its different phases vary little from the normal as a result of vagotomy. The slowing in C after the subsidence of the stage B is seen to be mainly due to the great increase (as compared to A) in the duration of the expiratory pause. Similarly the increased rate resulting from rebreathing is due, as under normal conditions, to a shortening of this phase.

The condition established after the immediate effects of section have passed off is as follows. The duration of the inspiratory phase is lengthened (from 1·5 to 3·0 sec. in the case figured) and, as in the normal animal, is slightly shortened with deep breathing (B and D). The 1st phase of expiration is little changed in C, but increases in duration rather more than would be normally expected with deeper breathing. The 2nd phase of expiration is much increased in length (2·7 sec. in A to 9·5 sec. in C). The increased frequency of breathing shown in D is solely due to the shortening of this phase, just as would be the case in the intact animal under similar asphyxial conditions, although it is evident that the chemical stimulus is less effective in shortening this phase after vagotomy.

It is particularly to be noted that the change from inspiration to expiration in curves B, C, and D, is as abrupt as in the pre-section curve A. There is no spasm or gradual passage from one phase to the other such as is described by Gad and to which he attached fundamental importance.

Schafer [1919], it will be remembered, states that if time be allowed for the subsidence of the initial effect of section the rate and depth of breathing return to about the normal. Trevan and Boeck [1922] indeed state that in animals in which decerebration has been effected in front of the anterior colliculi or in animals under light anæsthesia, section of the vagi is not followed by the usually described change in rhythm. The present writers, in dogs observed for varying periods up to 36 hours under the experimental conditions described, have always obtained the classical effects of section of the vagi. While the ventilation returns to about the normal, as regards the form of the cycle and the frequency of the rhythm they have generally seen the result illustrated in Fig. 2 B

and C. The larynx having been excluded by a tracheal cannula the results cannot be ascribed (as Schafer suggests) to laryngeal obstruction[1].

That great slowing of the breathing and some increase in depth (perhaps secondary to the diminished ventilation due to slowing) are the normal effects of removing the vagus influences affecting the centre and are permanent and not due to excitation at the point of section seems to be conclusively demonstrated by Pavlov's observation [1895, 1896] (for the reference to which the writers have to thank Prof. Anrep). That author found that in dogs in which both vagi had been cut, on one side below the origin of the inferior laryngeal nerve and on both sides below the origin of the superior laryngeal, the animals were in good health a year later, the breathing, however, varying from only 4 to 7 per min. The contention therefore that the absence of reflex vagus influences from the centre leads to an increase in extent and duration of inspiration and a considerable prolongation of the expiratory pause appears to be justified.

From the description given above of the normal cycle (p. 85) it is clear that the expiratory collapse does not initiate inspiration. The following observations give direct experimental proof of the accepted view that inspiration is terminated by the inhibitory reflex set up by expansion of the lungs.

If measurements of the time and volume relations in the four curves shown in Fig. 2 be made, it will be found that in cycle A inspiration terminates with a depth of 105 c.c. in 1·5 sec. If the expansion reached in 1·5 sec. (marked T on the curves) be measured, it will be found to be the same (105 c.c.) in C, and only slightly increased (115 c.c.) in B. Thus the time taken to reach a given depth of inspiration is the same after as before section when no asphyxial element is present. In D, in which the chemical stimulus was increased, an expansion of about 210 c.c. was reached in the same time. The degree of expansion of the lungs reached in any given time is clearly governed by three factors, the force of the discharge from the centre, the duration of the discharge (period of muscular contraction), and the frictional resistance to the air entry.

The force of the discharge (or its rate of development) from the centre seems to be the same in B and C, after, as in A before section.

[1] The writers have compared the effects of different methods of blocking the nerves, namely by cutting, freezing and the injection of cocaine solution into both vagi near their exit from the thorax. The results have been the same in each case; suggesting that excitation at the point of section is not the cause of the slow deep breathing. Barcroft and Margaria [1931] observe the same initial increase, in ventilation with return to approximately the normal due chiefly to a diminished amplitude of inspiration.

It does not seem possible to explain this observation except by supposing that had the vagus been intact in B and C the discharge would have been cut short when inspiration had reached the pre-section depth by an inhibitory impulse which at that point balanced in strength the force of the discharge, but that in the absence of the vagus reflex the discharge continues for its normal unchecked term.

In cycle D in which the force of the discharge was increased by the increased chemical stimulus, taking the time 1·5 sec. as a measure, the inhibition would have become effective when the expansion had reached about 210 c.c. This may possibly have been the depth which inspiration would have reached under the same asphyxial conditions with the vagi intact instead of the maximal expansion of 550 c.c. actually observed.

Fig. 11 B may be compared in which with excess CO_2 the depth of inspiration in a dog of similar weight (without section of vagi) did not exceed 220 c.c., except for occasional gasps reaching 450 c.c.

Assuming the interpretation of this observation to be correct it is evident that whatever the increase in the chemical excitability of the centre may be, with the vagi intact, the discharge is cut short and the expansion of the lungs never maximal. [The advantage of this is that the time taken in filling and in emptying the lungs is less (for mechanical reasons) than would be the case if the expansion were unrestricted.]

For reasons given above (p. 88) the effects of vagotomy cannot be regarded as excitatory in origin. The fact that section of one vagus produces little effect on the breathing (a stimulus applied to one nerve being as effective as simultaneous excitation of both) implies that even the initial effects of section of both vagi are due to the cutting off of influences arising in the lungs. It has already been shown (p. 87) that the response in frequency of the centre to an excess of CO_2 is diminished after vagotomy: Scott [1908], indeed, believed that this response to asphyxial conditions was lost. It thus seems clear that some influence which normally facilitates the rate of discharge from the centre is cut off.

It had not been intended to discuss further the origin of these initial changes. It has, however, been suggested since this paper was written that the slowing following section is sufficiently explained by the diminished chemical stimulus to the centre resulting from the increased alveolar ventilation. The point is of fundamental importance, as it would imply that the rate as well as the force of discharge from the centre is governed solely by the chemical composition of the blood, and that the nervous influence from the lungs is confined to the regulation

of the degree of inspiratory expansion. The following table gives data from observations figured later in this paper (pp. 97, 107), which indicate that some influence additional to the chemical is required to account for the facts.

TABLE I. Changes in rate and depth of breathing after vagotomy.

	Observation I			Observation II			Observation III		
	A	B	C	A	B	C	A	B	C
1. Before section	36	48	1730	18	75	1350	42	60	2520
2. 1st vagus cut	36	75	2720	21	120	2520	36	68	2448
3. 2nd cut (initial effect)	12	280	3360	10	188	1880	—	—	—
4. „ later	12	340	4080	7	340	2380	8	125	1000
5. „ later	—	—	—	7	200	1400	8	87	696

Observation I from Fig. 5 A; observation II from Fig. 5 B; observation III from Fig. 12 B. *A*, rate per min.; *B*, mean depth of inspiration in c.c.; *C*, ventilation per min. Observation I: between parts 3 and 4 interval of 3 min. Observation II: between parts 3 and 4 interval of 3 min.; between parts 4 and 5 inflation experiment, after which animal breathed to open air for a few minutes. Observation III: between parts 3 and 4 interval of 35 sec.; between parts 4 and 5 a few minutes with breathing to open air. In observation II, Fig. 5 B, part 5 not shown in figure. In observation III, only part 5 shown in Fig. 12 B. Observation II, part 3 mean of 7 cycles, part 4 mean of 5 cycles.

It is from the conditions established by section of the 1st vagus that the effects of the 2nd section start and with which these latter effects must be compared.

In observation I the slow rhythm after section of the 2nd vagus might be explained as being the result of the increased ventilation. In observations II and III the greatly decreased rate accompanied by decreased ventilation cannot be explained in this way. Even if the initially increased dilution of the alveolar air at the beginning of each cycle be taken into account the mean alveolar ventilation must have fallen as the immediate effect of section.

Some adaptation occurs after a varying time (Fig. 2 C and observations II, 5 and III, 4). It is entirely at the expense of the depth, the frequency remaining constant. If the deep breathing following section were responsible for the decreased rate a diminished depth with much decreased ventilation (per breath) would be expected to cause an increase in rate. As seen from Figs. 5 A and B this does not occur. In 5 A inspiration is resisted by deflation, in B expiration by inflation. In neither does the rate change. In 5 B, for example, it will be seen that at the 4th cycle after the commencement of inflation, when the depth has decreased from 340 to 75 c.c., the duration of the cycle is unchanged at 7 per min. That this is not due to the resistance is shown by the fact that the rate (slightly increased by excess CO_2) just before release of the

inflating pressure is identical with that immediately after when the volume was again about 340 c.c.

It seems impossible to explain these facts except by supposing that the vagus conveys an influence which renders that part of the centre concerned with rhythm more responsive to the chemical stimulus. This influence is accelerator in the sense that in its absence the rhythm is slowed. (Rosenthal [1862] held this view on other grounds.)

Summary of the results observed in animals under the experimental conditions described (Section I).

1. The duration of inspiration and the 1st phase of expiration are little changed by changes in rate or depth of breathing. Changes in the rate are due to an increase or decrease in the duration of the 2nd phase of expiration. This is also true of the rhythm after vagotomy.

2. The 2nd phase of expiration shows no evidence of any change in the volume of the lungs which could excite inspiration or inhibit expiration.

3. The form of the cycle after section of the vagi is comparable to the normal. The inspiratory phase is longer but terminates with the normal abruptness, no sustained inspiration as described by Gad being seen. The permanent slowing is due mainly to the prolongation of the expiratory pause

4. The time taken to reach a given degree of expansion of the lungs is approximately the same after as before section of the vagi under similar conditions of ventilation.

Appendix to Section I.

Since this paper was completed the attention of the writers has been drawn to the need of some explanation of the fact that the form of the cycle described differs from that usually regarded as normal for man.

That the form described is not related to the anæsthetic and is not peculiar to the dog is proved by the following facts.

(*a*) The form is the same in decerebrate animals in which chloralose was not used. (*b*) In the cat used in demonstrating the method at the Meeting of the Physiological Society in July, 1928, decerebration was carried out 4 hours before the experiment; no morphine or chloralose had been administered. The form of the cycle was identical with that described. (*c*) Gad and Head using rabbits under chloral give the same description of the cycle. Gad, in fact, found that this form of the cycle was normal for the unanæsthetized rabbit after the emotional disturbance incidental to the experimental procedure had subsided and the animal had become quiescent.

It is of interest to note that Gad's description of the initial (emotional) cycle is very similar in form to the type usually regarded as normal for man.

In the cycle, as usually observed in the human subject, by any method of recording, the duration of the inspiratory and expiratory phases is not very different, the 2nd phase of expiration (the pause) being absent.

Barcroft and Margaria, in a recent paper [1931], from observations on themselves (recording with a spirometer of the Krogh type), find the inspiration occupies about one-third of the whole cycle, and that, contrary to what the present writers have observed in the dog, with increased rate of breathing accompanying exercise or an excess of CO_2 in the air, the shortening of the whole cycle is due to a proportionately equal decrease in the duration of both inspiratory and expiratory phases.

While it is true that the form of the cycle in man is that mentioned above, a type is not infrequently seen which closely resembles that described in this paper.

This may be observed in Haldane and Priestley's observations with the body plethysmograph [1905]. In Fig. 4 of their paper the more usual type is seen. In Fig. 3 some of the cycles show a well-marked 2nd phase of expiration. And in Fig. 5 all the cycles would, if registered with a faster recording speed, have resembled closely that characteristic of the dog. Luciani, in his *Text-book of Human Physiology* (Vol. I, 1911), gives two figures which are particularly instructive. Fig. 184 on p. 418, from observations by Mosso, shows the form of the cycle in the same man awake and when asleep. In the first the form is similar to that generally described, in the second the inspiratory phase is rapid and is followed by a short 1st phase of expiration and a prolonged 2nd phase, the line of which is roughly horizontal.

Mosso ascribed the difference to the preponderance in the former of the abdominal (diaphragmatic) element in the breathing. The same type, resembling that described by the present writers, is seen in Fig. 185, p. 420. It represents the cycle during quiet breathing in the human subject. In the cases quoted the inspiratory phase appears to cover rather less than one-quarter, the pause rather more than one-half the whole period of the cycle.

The experimental conditions described in this paper differ in the following essential particulars from those usual in observations on man:

(1) A mouthpiece, nose-clip and valves are absent. (2) The normal frictional resistance to the flow of air (increased during expiration) through the larynx and upper air passages is eliminated. (3) The possible influence of stimuli arising in the sensory nerves of the larynx, pharynx and nasal mucosa is cut out. (4) The effect of influences originating in the cerebrum is absent.

Expiration appears to be normally a passive act of recoil, the force exerted being in quiet breathing probably not more than sufficient to overcome a pressure of about 5 mm. Hg at the beginning of expiration. It is evident that the form of the expiratory phase would be altered in the direction of a more gradual slope by any resistance to the passage of air. It is of interest in this connection that in the examples given from Haldane and Priestley and from Luciani no apparatus obstructed the normal passage of air through the mouth and nose. Also that, according to Semon (quoted from Luciani), in a large proportion of persons during quiet breathing adduction of the vocal chords does not occur during expiration, the glottis being wide open during the whole cycle.

The complicating factor of sensory consciousness remains, the effect of which may vary greatly in different individuals.

It would be outside the scope of this paper to discuss the various modifying influences (reflex or cortical) which must in the conscious animal, particularly in man, be necessary for the adaptation of the rhythm of breathing under varying conditions of posture or bodily movement.

That such influences exist and must form a normal part of the mechanism regulating the breathing cannot be doubted.

In the dog under the experimental conditions described in this paper the centre is isolated from other than reflex influences, of which those arising in the lungs are the most important. These must constantly be present and must underlie the modifications of the breathing resulting from influences of other origin.

Section II.

The reflex effects of expansion and collapse of the lungs.

Briefly stated the observations recorded below show that, starting from the normal volume of the lungs at the end of expiration the effect of artificial expansion is to cause a slowing or more or less prolonged suspension of respiration. The duration of the pause is dependent on and exactly proportional to the increased pulmonary volume, that is to the alteration in the position of equilibrium of the lungs and their surroundings brought about by the expanding force. Where the change in volume is slight this leads simply to a slower rhythm clearly due to a prolongation of the 2nd phase of expiration. The more prolonged pause, the well-known effect of inflation, must it seems be regarded as an exaggeration of this phase, brought about, it might be assumed, by a change either in the direction of a decrease or increase of the same reflex influences which, in addition to the chemical stimulus, govern the duration of the normal pause and so the rate of breathing.

Collapse of the lungs beyond the normal position of equilibrium is accompanied by an increase in the rate of breathing proportional to the diminution in lung volume. Just as acceleration from other causes is due to a shortening of the expiratory pause, so, in this case, the increased rate is primarily due to the same change in the cycle.

The effects produced by inflation and deflation respectively are in fact opposite phases of the same reflex origin. Whether an increase or decrease in the rate of breathing occurs, depends merely on the level from which the change in lung volume begins.

It will be convenient to illustrate the effect of considerable changes in volume of the lungs by the description and discussion of an observation lasting 12 min. continuously. The record of this observation is reproduced in Fig. 3.

Effect of inflation. It will be seen from the record in Fig. 3 that the different degrees of inflation shown in the observations marked 2, 3 and 4 are accompanied by a complete cessation of respiration, a pause the duration of which, on comparing the figures given in columns 3 and 5 of the table, is evidently almost exactly proportional to the increased volume of the lungs and the negative pressure by which it was produced.

It will be noted that there is no indication of any immediate expiratory response to expansion nor indeed of any immediate increase of expiratory

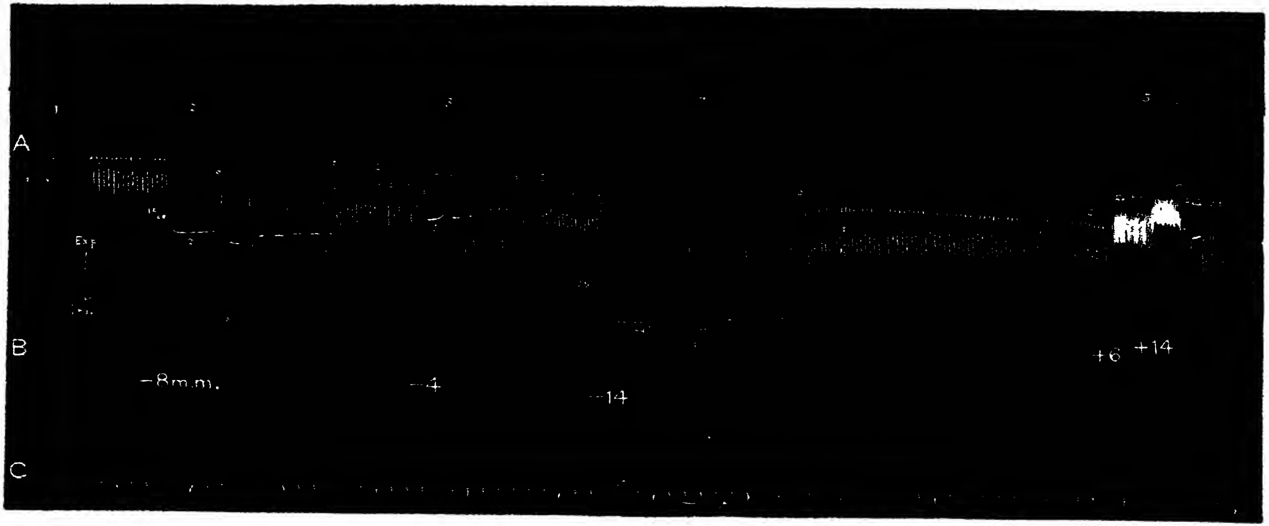


Fig. 3. The reflex effects of inflation and deflation (continuous record lasting 12 min.). Dog 14, wt. 6 kg. Morphine C.E. Chloralose 0·4 g. A, bellows recorder. B, manometer (Hg) showing change of pressure in chamber. C, time marker 10 sec. Obs. 1, normal cycle with medium fast rate of kymograph. Obs. 2, 3 and 4, inflation with negative pressure of 8, 4 and 14 mm. Obs. 5 and 6, deflation with negative pressure of 6 and 14 mm. Hg.; *O*, opening, *C*, closing of cock releasing pressure in chamber. Read from left to right. Inspiration down, expiration up. The following table shows results in the order of progressive increase in lung volume from the minimum obtained with deflation.

Observation	Pressure in box (mm. Hg)	Change in volume of lungs (c.c.)	Breathing rate (per min.)	Duration of pause (sec.)
6	+14	− 74	93	—
5	+ 6	− 40	50	—
1 (initial)	0	0	18	2·2
3	− 4	+ 75	—	13·0
2	− 8	+150	—	27·0
4	−14	+250	—	39·0

tone, either of which might have been regarded as a reflex effect of expansion of the lungs. In observation 4, after a lapse of 39 sec., and proportionately earlier in observations 2 and 3, there appears a rapidly increasing active expiratory effort not corresponding to anything occurring in the normal cycle, but possibly representing the 3rd phase of expiration described by Head mentioned above (p. 84). The expiratory phenomena accompanying inflation will be discussed later in this paper, when evidence will be adduced that such phenomena are not of pulmonary reflex origin.

The pause must, in fact, be regarded as the establishment of a position of equilibrium during which no active inspiratory or expiratory forces are in action.

Effect of deflation. In observations 5 and 6 (Fig. 3) positive pressures of 6 and 14 mm. Hg respectively were established in the chamber. The effect was to cause an immediate increase in the rate of the breathing to 50 and 93 per min. in the two cases, the rate remaining constant as long as the deflation was maintained at the same level. It will also be seen that the increased frequency is proportionate to the decrease in lung volume.

These two points are shown more precisely in the figures tabulated below from other experiments.

TABLE II. Effect of deflation in two dogs *A* and *B*. The results are given per min. The duration of each stage was about 30 sec.

Pressure mm. Hg in chamber	Decrease in c.c. in lung volume	Rate per min. of breathing	Depth in c.c.	Ventilation per min.
A. Atm.	0	15	80	1200
+ 3 mm.	−23	21	80	1680
6 ,,	−42	24	77	1848
13 ,,	−70	30	59	1770
B. Atm.	0	12	66	792
+ 8 mm.	−55	24	77	1848
6 ,,	−45	20	73	1460
8 ,,	−55	24	66	1584

The effect of rapid and complete collapse of the lungs is shown in Fig. 4.

Rapid collapse of the lungs was produced, as shown in the figure, by a positive pressure in the chamber of 90 mm. Hg in 17 sec., the reserve air (75 c.c.) being expelled and the lungs reduced to their minimal volume (a volume they would have reached if removed from the body) in 10 sec.

It is to be noted that, just as in Fig. 3 (5 and 6), there is no indication of any inspiratory response to collapse, such as has been usually described.

It will be observed that in observations 5 and 6 (Fig. 3) and in observations *A* and *B* (Table II) the increased frequency accompanying deflation is accompanied by increased ventilation, thus in the former the ventilation per min. which was 2100 c.c. before deflation, was raised to 3500 in observation 5 and 5600 in 6. That diminished alveolar ventilation due to decreased depth of inspiration cannot account for the accelerated breathing is demonstrated by the fact that in the observations of which the data are given in Table II, the depth of inspiration is the same or slightly increased. The sustained frequency without apnœic slowing in spite of the increased exchange of air appears to show that collapse of the lungs is accompanied by a reflex increase in excitability of that part of the central mechanism controlling the rhythm of respiration (see also, pp. 89 and 90 of Section I above).

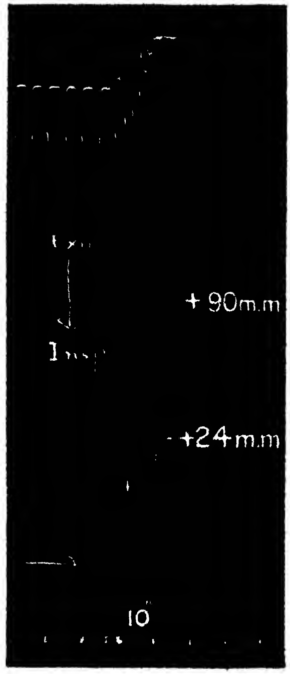


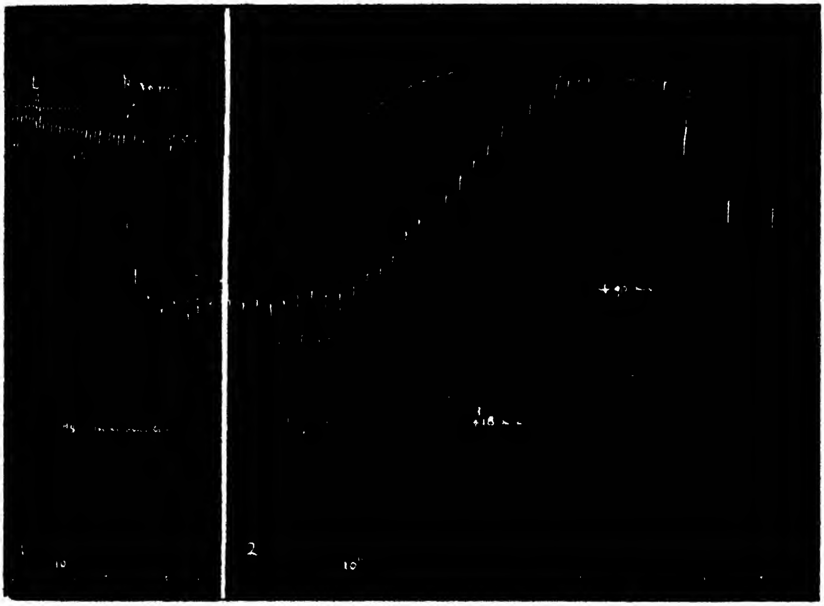
Fig. 4. To show effect of sudden deflation. Dog IV (1). Chloralose. Initial rate of breathing 12 per min. Pressure was raised in box to 90 mm. Hg in 14 sec. The limit of collapse of the lungs was reached in 8½ sec. at 24 mm. pressure; in 8 sec. there were 5 inspirations = 36 per min. Air expelled from lungs at limit of collapse was 75 c.c., representing the reserve air. There is no sign of an inspiratory reaction to collapse. Record as usual. Inspiration down. Read from left to right.

That the effects of inflation and deflation are reflex phenomena originating in the lungs is shown by Fig. 5, in which A illustrates the effect of expansion, B of collapse of the lungs, after section of the vagus nerves.

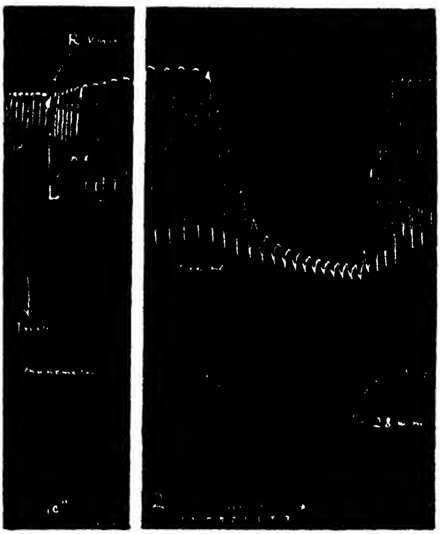
It will be seen at once that in neither A nor B was there any change in frequency, although the change in volume was considerable in both directions.

Threshold changes of volume. Fig. 6 shows the results accompanying inflation by negative pressures of − 2·5 and − 4 mm. Hg.

It will be seen from the record that these small degrees of inflation produce a well-marked slowing of the rhythm. In the observation with a negative pressure of 2·5 mm. the increase in volume of the lungs, measured from the original base line of expiration, was approximately no more than 30 c.c. It is quite clear, from an inspection of the tracing, that the pause, which varied in duration in the different cases from



A



B

Fig. 5. To show the effect of inflation and deflation respectively after section of the vagi. A, effect of deflation. Dog IV (1). Morphine. Chloralose. Left vagus cut at *L*. Right at *R*. Air pressure in chamber was raised gradually to 90 mm. Hg. At 18 mm. the lungs were compressed to their minimum volume. No acceleration of breathing is seen. Short interval between parts 1 and 2 of tracing. B, effect of inflation. Dog V (1), wt. 4 kg. Morphine. Chloralose. Right vagus cut at *R*. Left at *L*. Pressure in the chamber was lowered to −28 mm. Hg. No change in the rate occurred. There is an interval of 3 min. between parts 1 and 2 of tracing. In both A and B read from left to right. Inspiration down, expiration up.

8 sec. to 22 sec., is simply an extension of the 2nd phase of expiration which had, in the normal breathing immediately preceding the observation, a duration of 2·5 sec.

It is to be noted that the depth of inspiration is unaffected by the sustained expansion. (There is a slight later increase from diminished ventilation.) The active expiratory phenomena, seen for example in observation 3, Fig. 3, with a similar amount of inflation, are not present, nor in fact do these expiratory reactions occur in all cases.

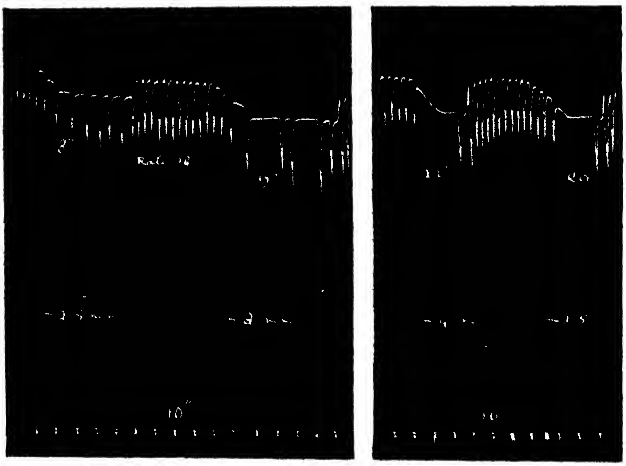


Fig. 6. To show the effect of slight degrees of inflation. Dog II (2). Morphine. A, C, E. Chloralose. Initial rate 20 per min. T.A. volume 70. Duration of expiratory pause approximately 2 sec.

Inflation with − 2·5 mm. Hg caused slow rhythm. Pause increased to 8 sec.
Inflation with − 3·6 mm. Hg, pause increased to 17 sec.
„ − 4·0 „ „ 22 „
„ − 3·5 „ „ 20 „

Inspiration down. To be read from left to right.

Effect of short terminal resistance to expiration. The fact that the same slowing of the rhythm occurs, resulting from a slight shifting of the position of equilibrium of the lungs, is shown by the following procedure in which no artificial inflation was induced, but in its place a small elastic resistance to collapse.

It will be remembered that in the apparatus used the animal lies in a closed air space, its lungs only being connected to the outer air (or bellows). The air space can be opened to the outer air through a release cock. If this be closed at the end of expiration the inspiratory expansion

of the thorax immediately following will cause a rising positive pressure in the chamber; conversely, closure at the end of inspiration causes an increasing negative pressure during expiration.

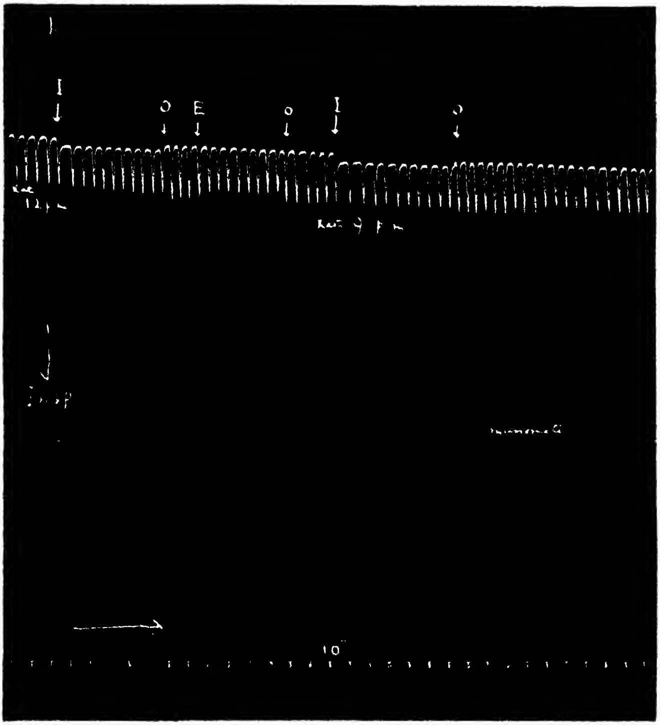
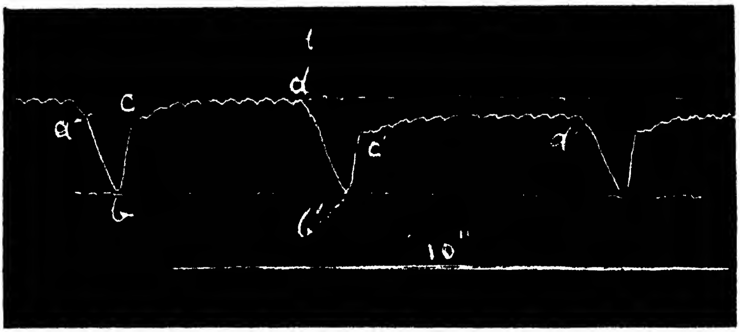


Fig. 7. To show the effect of a slight terminal resistance to expiration and inspiration respectively. Dog II (2). Initial rate 12 per min. T.A. volume 70 c.c. *I*, closure of release cock (connecting interior of chamber with outer air) at end of inspiration. *O*, opening of cock. *E*, closure during expiratory pause. Closure at *E*. No change in rate. Base line of expiration unchanged. Depth of inspiration reduced from 75 c.c. to 60 c.c. Terminal resistance to inspiration = 1·5 mm. external pressure. Closure at *I*. Rate reduced to 9 per min. Base line of expiration changed 15 c.c. in direction of expansion. Volume of lungs reached in inspiration unchanged. Terminal resistance to expiration = external negative pressure of 1·5 mm.

The change in pressure (from atmospheric at the moment of closure) is in the ratio of the volume of the tidal air (60 to 100 c.c. in the dogs used) to the volume of the air space around the animal which was about 20 litres.

An increasing elastic force is thus introduced opposing the elastic forces of recoil in expiration or the muscular force of inspiration. Provided therefore there is no change in either of these factors a new level of equilibrium will be reached in expiration and similarly in inspiration. This is what happens, expiration being restricted in the one case, inspiration in the other.

The mode of observation is that the observer, watching the recording point of the bellows with his hand on the key of the release cock, closes the latter at any selected point in the cycle.



↑ ↑

Fig. 8. Effect of slight terminal resistance to expiration, showing the phases of the cycle. Dog I (2). Recording with medium fast rate of kymograph. Normal cycle *a*, *b*, *c*, *d*. The release cock was closed at *b'*. Upper dotted line indicates original base line of expiration. Lower dotted line through *b*, *b'* level reached in inspiration. Duration of the cycle *a*, *b*, *c*, *d* before closure 6 sec. Duration of the cycle *d*, *b'*, *c'*, *d'* 7·2 sec. Duration of expiratory pause before closure *c*, *d* 4·8 sec. Duration of expiratory pause after closure *c'*, *d'* 6 sec. The duration of the inspiratory phase and recoil phase of expiration was the same, 1·2 sec. in the three cycles shown. Original depth of breathing 66 c.c. Change of volume from *c*, *d* to *c'*, *d'* was 11 c.c.

Fig. 7 illustrates an observation of this kind in which a slight terminal resistance to expiration is introduced at *I*, and a slight resistance to inspiration at *E*.

It will be observed that when the cock is closed at the moment when the lungs have reached their full expansion in inspiration, *I* in the figure, there is an immediate lowering of the base line of expiration (in the direction of expansion) accompanied by a diminution in frequency from 12 to 9 per min. The original tidal air volume was 70 c.c.; this was reduced to 55 c.c. by the effect of the gradually increasing negative pressure which, at the new base line, was equal to – 1·3 mm. Hg. It

will be seen that there is no change in the level at which inspiration comes to an end.

In the reverse procedure, in which the cock is closed at the end of expiration, at *E*, a change in the depth of inspiration is produced of about the same amount as in expiration in the first case; it will be observed that there is no change in the position of the base line of expiration and no change in the frequency.

The form of the cycle and the above-mentioned facts are well shown in the observation, carried out in the same way, recorded in Fig. 8.

In the figure *a*, *b*, *c* and *d* represent the original form of the cycle. At *b′* the cock was closed. It will be seen that the passive 1st phase of expiration *b′c′* comes to an end sooner than does *bc* in the normal cycle; the base line of expiration being thereby lowered by 11 c.c. The only change in the duration of any part of the cycle is in the 2nd phase of expiration *c′d′*, which was lengthened from 4·5 to 6·5 sec.

It will be noted that the level reached in inspiration is the same in both curves. The depth of inspiration has thus no influence on the duration of the pause.

That the slowing of the rhythm recorded in the figure is due to a vagal pulmonary reflex, accompanying the sustained change in the expiratory volume of the lungs, is demonstrated by the observation illustrated in Fig. 9, carried out by the same procedure after section of the vagi.

Fig. 9. Slight terminal resistance to expiration after vagotomy. To compare with Figs. 7 and 8. Closure of cock at *I*, end of inspiration. Opening at *O*. Base line of expiration lowered 60 c.c. No change of rate of breathing.

The cock was closed at *I* at the end of inspiration and opened again at *O*. The negative pressure resisting expiration, at the level at which the new position of equilibrium was reached, was estimated to be 7·5 mm. Hg. In spite of the considerable shifting of the base line of expiration in the direction of expansion it will be seen that there is no alteration in the rate of breathing.

It is evident from the observations illustrated in Figs. 7, 8 and 9

that (*a*) a change in the position of equilibrium towards expansion at which the lungs come to rest causes a slowing of the rate of breathing due solely to a prolongation of the expiratory pause; (*b*) the level of expansion reached in inspiration has no influence on the duration of any other part of the cycle; (*c*) whatever may be the nature of the reflex influences, or the nature of the peripheral stimulus (such as tension), which reflexly influence the rate of rhythm, they must be of a tonic nature as they do not depend for their intensity on a changing state of expansion, but on a stationary position of the lungs at any degree of expansion; (*d*) the reflex influences which affect the frequency do not affect the depth of breathing.

These phenomena appear not to be the same as those described by Davies, Haldane and Priestley [1919], namely that increased frictional resistance to the entry and exit of air in the human subject slows the breathing owing to the prolongation of inspiration as well as expiration. In the procedure of which the results are recorded above (Figs. 7 and 8) there is no resistance to the passage of air, there is merely a slight diminution of the elastic force which determines the position the lungs return to in expiration. There is no change in the cycle except the entirely reflex lengthening of the 2nd phase (pause) of expiration. It may be pointed out that in the above authors' method the resistance to air exit exceeds the elastic force to which expiration is mainly due, expiration being thus converted from a passive recoil to an active muscular effort.

Response to volume changes with increased central excitability. Fig. 10 shows three successive observations on the same animal under different conditions. A, breathing normal air; B, breathing air containing an excess of CO_2; C, with air containing no CO_2 but deficient in oxygen.

(*a*) *The effect of excess* CO_2. It will be seen on comparing records A and B that in the latter the increasing excess of CO_2 leads (without any anoxæmic factor) to a progressive diminution in the reflex effect of inflation of the lungs until, at the 5th minute, the pause is replaced by a simple slowing of the rhythm. As under normal conditions there is an acceleration of the breathing with collapse, and attention is particularly drawn to the fact that, although the central excitability was evidently much above the normal, there is no evidence of any inspiratory spasm or hypertonus in response to the deflation of the lungs.

(*b*) *Effect of anoxæmia.* An inspection of record C (illustrating the effect of increasing anoxæmia with continuous removal of the CO_2) shows that anoxæmia alone is accompanied by a well-marked diminution in the

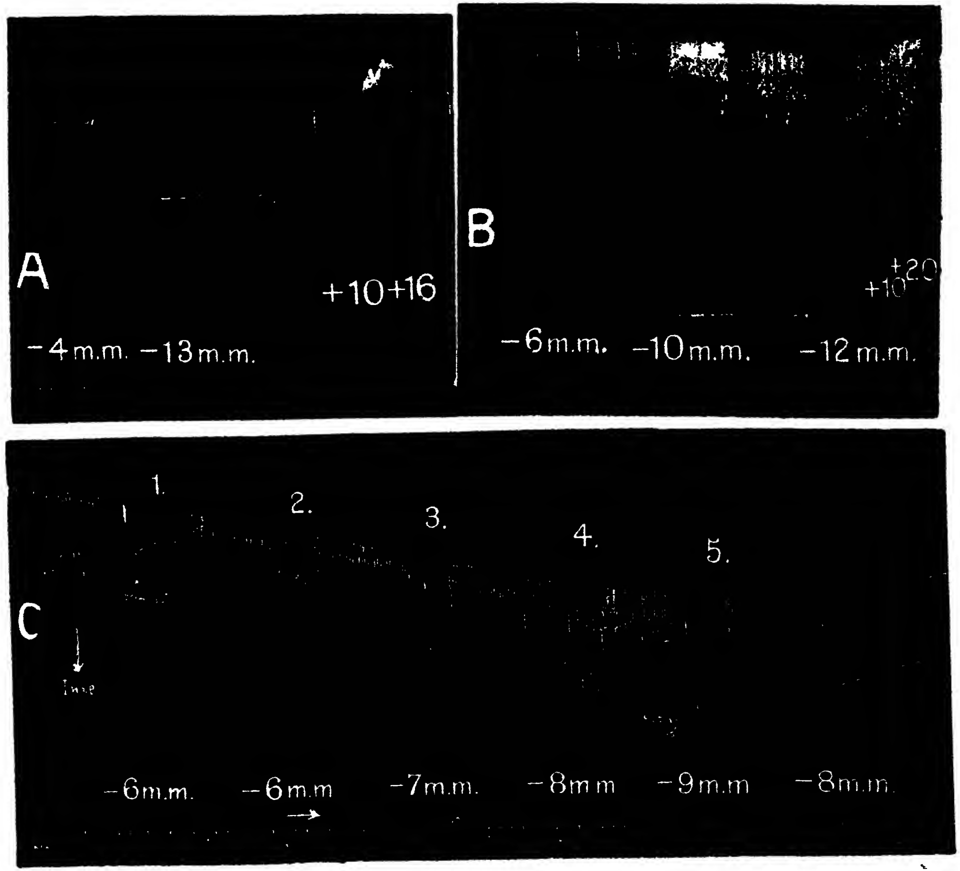


Fig. 10. Effect of asphyxial conditions on the response. Dog *F*, wt. 6 kg. Morphine 30 mg. Chloralose 0·45 g. A, normal. B, excess CO_2. C, anoxæmia. A, initial rate 24 per min. Vol. 66. −4 mm. pressure, pause 20 sec.; −13 mm. pressure, pause 62 sec. B, rebreathing from 1 litre bottle to which was added excess CO_2 6 p.c., excess oxygen 10 p.c. Total vol. air in bottle, bellows and lungs approx. 2200 c.c. Initial rate 15 per min., vol. 50. After 5 min. rate 42, vol. 220. After 75 sec., −6 mm. pressure, pause 9 sec. After 5 min., no pause but slowing at 24. C, rebreathing through inlet and outlet valves to 1 litre bottle with CO_2 absorber in circuit. Estimated O_2 content of system at 10th min. 4 p.c. Initial rate 16 per min., vol. 50. 10th min. rate 27, vol. 150. Effect: inflation −6 mm. pressure 2nd min., pause 30 sec.; 10th min. −9 mm., pause 12 sec. x in obs. I = end of inspiration. All records inspiration down. Read left to right. Time 10 sec. Minutes 1 to 13.

reflex inhibitory effect of inflation, although it is evident that this reflex effect is never diminished by a deficiency of oxygen to an extent comparable to that seen with an excess of CO_2. For example, at the 10th minute when the oxygen content of the air breathed was reduced to 4 p.c. and the ventilation increased five times a comparatively small expansion produced by a negative pressure of 9 mm. Hg still induced a pause lasting 12 sec.

If it be remembered, however, that the greatly increased ventilation, with complete removal of the CO_2, must have led to a washing out of that gas from the blood sufficient normally to cause prolonged apnœa, it is evident that anoxæmia alone has a strong excitatory effect on the central nervous mechanism.

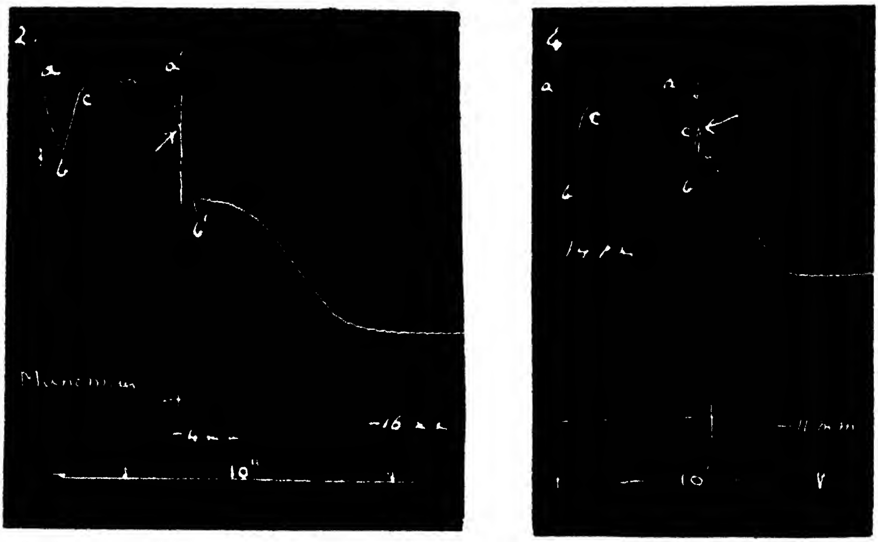
(*c*) *The effect of increased temperature.* In dogs and other animals in which heat regulation by evaporation takes place mainly through the lungs and air passages, an air temperature which does not cause (in anæsthetized animals) a rise of body temperature exceeding about 1·0° C. quickens the breathing to 60 or 90 per min. It was found that in this type of heat response the respiratory cycle conforms to the normal type and that the response to inflation diminishes, as in CO_2 excess, progressively as the rate of breathing increases. The acceleration is seen equally well after decerebration and vagotomy has the usual effect. The effect is evidently due to the rise of temperature increasing the excitability of the respiratory centre in the medulla or mid-brain.

If the body temperature rises more than about 1·0° C. above the normal, panting (heat polypnœa) commences, the rate rising suddenly to 200 or more per min. The form of the cycle is altered, inspiration and expiration being of equal duration. The normal reflex effects of inflation and deflation are entirely absent. Section of the vagi has no effect. The depth of breathing may be normal, ventilation being increased fifteen times, in some cases. Panting does not occur after decerebration immediately in front of the anterior colliculi. The phenomenon seems to be due to the effect of heat on a centre lying above the mid-brain which supersedes the central mechanism normally controlling the rate of breathing.

The effect of inflation beginning during different phases of the cycle. The six successive observations recorded in Fig. 10 C (anoxæmia) show a point of some interest. It will be noticed that in the first observation (at the 2nd minute) there is a downward peak, *X* in the figure, caused by a sudden return in the direction of expiration. Attention is drawn to this, as the appearance is similar to that often described and sometimes

regarded as evidence of an active expiratory response to expansion. The recoil is, however, a passive effect due to the sudden cessation of assistance given to expansion by the inspiratory muscles: the moment these cease to contract the various elastic forces opposing inflation come into play.

The phenomenon only occurs if the artificial inflation begins during the inspiratory phase of the cycle; it happens to do so in this case. In



A B

Fig. 11. Effect of inflation beginning during different phases of the cycle. Dog. Morphine and chloralose. *a*, *b*, inspiratory phase; *b*, *c*, expiratory 1st phase. A, inflation at the arrow about half-way through phase *a′b′*. Inspiration continues to *b′*. Duration of *ab* and *a′b′* the same = 1 sec. B, inflation half-way through recoil phase at arrow. No delay.

the 2nd, 3rd, 5th and 6th observations, in which inflation begins during the expiratory phase, the peak is absent.

The fact is better shown by the two observations illustrated in Fig. 11.

In A inflation begins when inspiration is about half completed, in B during the first phase of expiration.

It will be seen from the figure that whereas in A the inflation is carried considerably beyond the point at which equilibrium is eventually established, a peak due to the combined effect of the continuing

inspiratory discharge and the inflating negative pressure resulting, in B the expansion follows exactly the form of the manometer tracing (lower line), there being no evidence of any recoil, active or passive. A number of observations, similar to the two illustrated, show that at whatever point in the inspiratory phase inflation begins inspiration completes itself and has the normal duration, the apex of the peak representing the termination of the act. The peak is not due to instrumental vibrations.

The expiratory phenomena accompanying inflation. The expiratory phenomena have been already referred to. They have been commonly regarded as evidence that expansion of the lungs gives rise to an expiratory response. They consist essentially of a gradual increase in expiratory tone culminating in an active effort terminated by an inspiration (see Figs. 3 and 10).

The form and extent of these reactions vary considerably in different experimental animals; occasionally they are absent (see Fig. 7), in others a definite rhythm is established in which expiration becomes the active phase, inspiration being almost entirely passive.

The writers have not yet attempted to investigate the conditions which favour the appearance of this reaction to inflation. Gad regarded their occurrence in Hering and Breuer's experiments as due to the use of opium as an anæsthetic by those observers. Head, as mentioned above, found that they represented forcible contractions of the abdominal muscles.

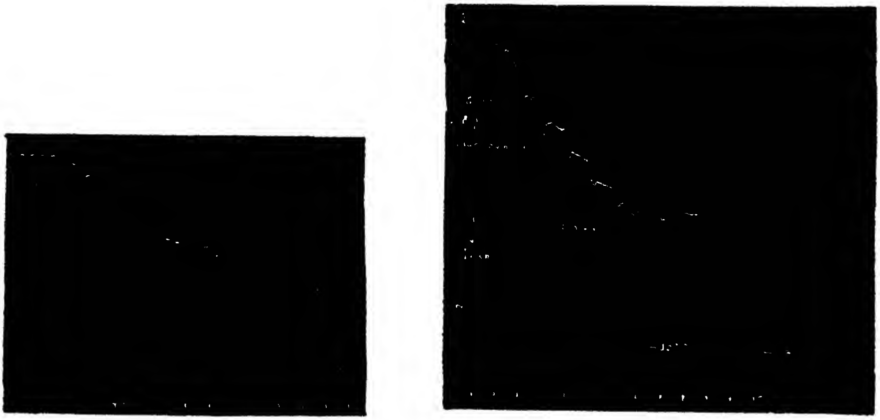
They are not apparently related to an increase in the chemical excitability. It may be noted, for example, that they are well seen in observation C of Fig. 10 at a time when there was only a slight deficiency of oxygen and a complete absence of CO_2 in the inspired air. In the writers' experience a well-marked degree of inflation is necessary for the production of these reactions, and they are much less marked in decerebrate than in chloralosed animals, possibly owing to a condition of shock depressing the tone of the abdominal muscles. It is possible that these phenomena belong to the group of extra-pulmonary proprioceptive reflexes described by Fleisch [1928, 1929].

That the origin of this expiratory reflex is not in the vagus terminations in the lungs but in nerve terminations in some other regions (*e.g.* the abdominal muscles) is indicated by the observations illustrated in Fig. 12, A and B.

Fig. 12 shows the effect of gradually increasing inflation: A, in an animal with the vagi intact; B, after section of both vagi.

If A and B in the figure be compared it will be seen, taking the dotted

line in both cases as showing the gradient which the expiratory pause would have followed if it had been entirely passive, that there is the same increase in expiratory tone in B as may be observed in A. It will also be noticed that there is a similar indication of a more active expiratory contraction immediately preceding inspiration. It is evident that owing to the shorter duration of the expiratory pause in B (following vagotomy) as compared to the duration of the pause (due to inflation) in A, the



A B

Fig. 12 A. Effect of gradually increasing inflation. To show increasing expiratory tonus during pause, effect becoming more marked with accumulation of CO_2. Dog IV (1). Morphine 10 mg. per kg. C. and E. chloralose 0·07 g. per kilo. Ventilation per min. before inflation 1350 c.c. Ventilation in 1st min. during inflation 450 c.c. Dotted line pressure gradient.

Fig. 12 B. For comparison with A. To show rise of expiratory tonus accompanying inflation after vagotomy. Dog. Morphine 10 mg. Chloralose 0·1 g. per kg. Rate of breathing before section 42 per min., vol. 60. Right vagus cut first. Rate 36, vol. 68. After section of left vagus rate fell to 8 per min., vol. increased to 125. After interval of breathing to open air vol. fell to 87 c.c. Rate unchanged at 8. Gradual inflation pressure fell to −30 mm. in 90 sec.

expiratory reaction in the former cannot be expected to be as marked as in the latter.

Summary (*Section II*).

1. Volume changes of the lungs from any given position of equilibrium cause, with expansion, a prolongation of the expiratory pause or prolonged suspension of breathing, with collapse increased frequency, the effect in each case being proportional to the change in volume. The depth of inspiration is not significantly affected.

2. A slight change in the position of equilibrium, in the direction of expansion, which the lungs reach at the end of expiration without any change in the level reached in inspiration, produces at once a reflex slowing of the breathing, indicating that the expansion occurring in inspiration has no after-effect.

3. The effects of inflation and deflation are abolished by vagotomy.

4. The reflex effect of inflation is diminished in proportion to the increased frequency and depth of breathing in asphyxial or other conditions which increase the excitability of the centre.

5. There is no evidence of an immediate active expiratory response to expansion or an inspiratory response to collapse implied in the Hering-Breuer hypothesis.

6. The slow increase in expiratory tonus and active expiratory efforts accompanying considerable inflation are reflex effects which do not originate in the lungs, analogous phenomena being seen with inflation after section of the vagi in the neck.

Discussion.

The observations recorded in this paper do not, it will be seen, entirely support the hypotheses of different observers outlined in the brief historical review given in the Introduction.

While that part of the Hering-Breuer hypothesis dealing with the reflex termination of inspiration is confirmed, the effects of inflation and deflation described do not support the fundamental statement that each phase of the cycle automatically controls its own duration and initiates the next. Nor do the observations support either Gad's view of the entirely inhibitory nature of the vagus control, or Head's, that in addition to the inhibitory influence arising during inspiration (expansion) with its after-effect determining the duration and therefore the rate of expiration, there is an opposite effect originating in collapse exciting the inspiratory discharge. Head's view in regard to this latter influence would imply that it would increase the force and duration of the inspiratory discharge from the centre to the spinal nuclei rather than the rate of the rhythm. The present writers' observations show that, apart from changes in the chemical stimulus, just as the duration of the pause is unaffected by the depth of inspiration, so the latter is uninfluenced by the duration of the pause or in fact by nervous influences, except in so far as it is controlled by the inhibitory reflex set up by the act itself.

It will be suggested later that this and other facts imply that the central mechanism is composed of two functional parts, a pacemaker

(pneumotaxic centre) controlling the frequency, but not the force of the discharge from a lower centre, and an influence arising in the lungs which maintains the tone of the former.

Although the majority of observers have assumed that the vagus reflexes from the lungs (set up by changes in volume) are due to the changing tension of the lung tissues, this view has not been universally accepted. Stefani and Sighicelli [1888] and Lumsden [1923] ascribed the effects of inflation and deflation to changes in intrapulmonary pressure. Lumsden believed also that stimuli set up by air currents moving over the mucosa of the air passages were the source of reflex control of the breathing. These views are negatived by the fact that in the experiments described there is no change of intrapulmonary pressure, and that during the 2nd phase of expiration there is no movement of air.

Previous observers have produced expansion and collapse by inflation of the lungs with air under pressure or by suction respectively. The phenomena accompanying expansion with or without change in the intrapulmonary pressure appear to be the same. In regard to the effects of collapse this is not so certain. Former observers using the older method (deflation by negative intrapulmonary pressure) have found an active inspiratory response and an increase of inspiratory tone, no evidence of which has been observed in deflation by an external positive pressure. That the earlier result may have been due to the reflex effect of diminished pressure in the air passages is indicated from an observation recorded by Hering and Breuer (*loc. cit.*) who state that if after puncture of the pleura air is sucked from the trachea an active inspiratory response occurs similar to that produced by collapse, although it is evident that little change can take place in the lung volume.

The large increase in ventilation with the accelerated rhythm accompanying deflation, and the prolonged pause with inflation, indicate that changes in the composition of the air within the lungs or of the blood acting on the centre are not concerned in the causation of these reflexes.

From the above it must be concluded that the reflex effects of volume changes are due to changes in the shape or tension of the lung tissues; there is, however, no evidence to show where the vagus terminations affected lie.

The results obtained in anæsthetized or decerebrate animals can only be obtained to a limited extent in the conscious human subject. It is not improbable that the reflex effects observed may normally be modified

or controlled by cortical influences. It seems, however, unlikely that reflex effects would appear under conditions of consciousness which are not seen under the experimental conditions described or that the reflex control of respiration in the dog differs in any essential manner from that of man.

The effects following section of the vagi have been discussed at some length above. It is clear that while the observations support the view that the depth of inspiration is governed by an inhibitory reflex balancing at some point the force of the discharge determined primarily by the chemical excitation of the centre, they also appear to show (p. 89) that by section an influence other than this is cut off from the centre which by its effect normally maintains a faster rhythm than would exist in its absence. In the absence of the vagi the disability suffered by an animal would thus be that under conditions when increased ventilation is required this becomes less and less effective owing to the centre failing to respond by a sufficiently accelerated rhythm.

Various observers [Lewandowsky, 1898, Alcock, 1905, Einthoven, 1908] have described an action current in the vagi during inspiration, and some a slighter electrical change with expiration.

Adrian more recently [1926], using the capillary electrometer with 3-valve amplification, has observed an electrical change indicating an excitatory state propagated along the vagus appearing and increasing during inflation of the lungs and continuing during sustained expansion. He finds, however, no evidence of electrical change during expiration or during sustained deflation. This author notes as the most significant result the complete absence of renewed discharge at the moment when the lungs reach their smallest volume in expiration (supporting the view that expiration does not reflexly excite inspiration).

Keller and Loeser[1] [1930], using a similar technique, confirm Adrian's observations, but report in addition that if the chest is opened and air sucked out of the lungs there is a renewed discharge when the lungs are fully collapsed. They consider that this may also occur in forcible breathing, although they find no discharge during expiration in normal breathing. They also give evidence of the existence of a slight state of tonic excitation of the vagus when the lungs are in the "neutral position" corresponding to the base line of normal expiration. They find an increase in this discharge with collapse of the lungs produced by suction; the intensity of the discharge depending, however, more on the rate of pressure change than on the extent of the change in volume.

[1] The writers have to thank Prof. Adrian for this reference.

These authors, in fact, regard the change of pressure as playing an important part in the sensory stimulation giving rise to the electrical changes in the vagus. Some of their results suggest that the excitations set up by their method correspond to the reflex inspiratory phenomena described by different observers as resulting from deflation produced by negative intrapulmonary pressure, not observed by the present writers, using a method in which no pressure changes occur within the lungs.

Although the increasing discharge with inflation may be regarded as representing the impulse giving rise to the inhibitory effects, it seems doubtful whether the increasing discharge through the vagus accompanying rapid changes of negative pressure in the lungs can be regarded as representing normal effects which might be regarded as responsible for the acceleration of the breathing described in this paper as resulting from deflation. That there must be some alteration in the vagal discharge when the lung volume is reduced is, however, evident from the fact that acceleration occurs.

It is suggested that while an inhibitory influence, the intensity of which varies with the lung volume, is certainly present there is also present an influence which maintains the tone of the centre (augmenting the frequency of discharge), but which remains in general at a constant level of intensity, being unaffected by changes in the tension of the lung tissues.

In regard to this the effects of excitation of the cervical vagus are inconclusive. While an increase in the rate of breathing has been described the most usual effects of faradization are inhibitory in nature, closely reproducing the effects of inflation of the lungs.

That some tonic excitatory influence is present seems, however, to be proved by the phenomena following vagotomy. It has been shown above (p. 90) that the great slowing of the breathing after section of the 2nd vagus can only be explained as being the result of the cutting off of some stimulus normally reaching the centre from the lungs. This stimulus evidently cannot be inhibitory in its effect, for if this were so at any rate an initial increase and not a decrease in frequency would result. The influence must therefore be of the nature suggested above, its action on the centre being to maintain a more frequent rhythm and a greater responsiveness, in this respect, to the chemical stimulus.

The results of inflation of the lungs are therefore to be ascribed to the progressively increasing inhibitory stimulus with submergence of the augmentor influence, and the effects of deflation to the progressive diminution of the inhibitory and the consequent increasing predominance

of the augmentor influence, the slow rhythm after section of the vagi being due to the cutting off of the latter.

The relation of the influences arising in the lungs to the central mechanism must be briefly considered in so far as the facts observed appear to supply any information.

Lumsden's subdivision [1923] of the respiratory centre is well known. He describes three anatomically and functionally distinct parts as concerned in the control, the pneumotaxic, the expiratory and the inspiratory (apneustic) centres. There is no evidence in the observations of the present writers to show whether the prolonged irregular inspirations (apneuses) can or cannot be justly regarded as phenomena representing the normal activity of the 3rd centre when uncontrolled by the 1st. In regard, however, to the expiratory centre, the form of the normal cycle shows no indication of any active expiratory reflex, and in the cycle after vagotomy there is no evidence of the spastic expiratory effects which should be present if Lumsden's views were correct.

Without considering further the validity of that author's views as to the nature of the activity of these centres, it is necessary to assume the existence of an upper centre (pneumotaxic) sending rhythmic impulses to a lower centre from which originate the inspiratory discharges to the spinal nuclei, the activity of both centres being equally affected by changes in the composition of the blood.

The facts described in this paper indicate that the excitability of the former is augmented by a tonic influence arising in the lungs, the effect of which is to cause it to maintain a more frequent rhythm and to respond more actively to an excess of CO_2, and that it is influenced in the opposite direction by the inhibitory stimulus whose intensity varies in proportion to the state of tension of the lung tissues.

The discharge from the lower centre to the spinal nuclei of origin of the motor nerves to the inspiratory muscles is cut short at any given point (depth of inspiration) by the inhibitory influence arising in the lungs during inspiratory expansion, the point at which this happens depending on the relative strengths of the discharge and of the inhibitory influence.

The intensity of the discharge (initiated by the rhythmic impulses received from the upper centre) appears to vary only with the intensity of the chemical stimulus and is not under reflex control from the lungs. The basis for this statement is that while the frequency of the rhythm is much decreased by inflation (*vide* Fig. 6) the depth of inspiration is unaffected except in so far as it may be increased by accumulation of

CO_2, and secondly that under similar conditions of ventilation the lungs reach the same degree of expansion in approximately the same time after as before section of the vagi.

Under normal conditions of breathing it would thus appear that the augmentor influence affects the upper centre, the inhibitory only the lower.

Conclusions.

The following inferences are drawn from the facts summarized at the ends of Sections I and II and the above discussion:

1. Two influences arise in the lungs which affect the breathing and explain the effects accompanying expansion and collapse of the lungs and the modified rhythm following vagotomy: (*a*) an augmentor influence affecting the frequency; (*b*) an inhibitory influence mainly affecting the depth of breathing; the former being unaffected by volume changes, the latter increasing with expansion, decreasing with collapse of the lungs.

2. The reflex automatic control of the breathing is confined to the limitation of the depth of inspiration by the inhibitory influence.

3. The function of these two influences, affecting respectively the rate and depth of breathing, is to maintain the most effective ratio between these factors in ventilation.

The writers have to thank Dr J. S. Haldane and Prof. G. V. Anrep for their valuable criticism and advice.

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DISCHARGES IN MAMMALIAN SYMPATHETIC NERVES.

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RECORDS of sympathetic impulses in the cutaneous nerves of the frog have been published already [Adrian, 1930; Adrian, Cattell and Hoagland, 1931], and when these were made it was found that slow impulses, presumably sympathetic, could be detected in the cutaneous nerves of the cat when the hairs were erected as a result of asphyxia. The present work deals with the persistent or "tonic" discharges which are found in mammalian sympathetic nerves and are chiefly vaso-constrictor in effect. Records have been made from the cervical sympathetic and from various nerves in the abdomen such as the hypogastric and the nerves running from the cœliac ganglia. For the cervical sympathetic we have used rabbits under urethane or chloralose and for the abdominal nerves, rabbits and cats.

METHODS.

The usual technique for recording nerve impulses was employed, but as the arrangement at present in use for mammalian nerves has not been described, a brief account may be given here.

The animal is placed in a large, double-walled, metal container which is earthed to the water pipes of the laboratory and acts as a screen against electric disturbances and also as an incubator. The space between the walls is filled with water and heated to 45° C. The floor of the chamber is covered with water and the front is closed by a glass panel hinged at the top to allow access to the preparation. The atmosphere in the chamber is warm and moist enough to keep an exposed nerve in good condition without frequent irrigation, but there is little or no trouble from deposition of moisture on the electrode leads which are fixed in the roof of the chamber. As the body of the animal must be insulated, it is placed on a wooden stand coated with hard paraffin wax, and this rests in a wooden framework which keeps it above the level of the water on the floor of the chamber. The electrodes are small glass tubes containing Ringer's fluid and plugged at the lower end with kaolin or

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gelatine. A spiral of silver wire coated with silver chloride dips into the Ringer, and contact with the nerve is made by a moist thread running from the kaolin plug or by a small paint brush filled with gelatine. As a rule, the nerve is cut distally, the input electrode is near the distal end and the earthed electrode proximal to it, but when it is desired to record the impulses without cutting the nerve three electrodes are used, the middle leading to the amplifier input and the two on either side leading to earth. With this tripolar arrangement potential changes in the body of the animal (*e.g.* from the heart) do not affect the electrode system, though it is impossible to tell whether the impulses are ascending or descending. Movements of the nerve (from pulsating vessels, etc.) are prevented by looping it over a small glass hook.

The amplifier used in the present work has five valves, resistance-capacity coupled, leading to the four pentode output valves of the Matthews oscillograph. The coupling condensers and grid leaks were chosen so that a steady potential difference would be reproduced as a deflection falling to half its initial value in 0·5 or 0·05 sec. In most of the records shown in the figures the amplifier is working at $\frac{1}{4}$ or $\frac{1}{8}$ of its maximum sensitivity. With $\frac{1}{4}$ sensitivity an input potential of 10 microvolts gives a deflection of 5 mm. on the recording surface.

The potential changes are viewed with a revolving mirror and converted into sound with a large cone loud speaker driven by a separate amplifier and giving adequate reproduction of bass notes. As the sympathetic impulses give very slow potential changes, they may be inaudible if the loud speaker has a poor response in the bass.

A few experiments have been made on cats previously decerebrated under chloroform and ether, otherwise the animals have been under full anæsthesia throughout.

General character of sympathetic impulses.

When a sympathetic nerve is dissected out, cut distally and placed on the electrodes, it is usually found that a succession of slow potential waves passes down the nerve as long as the animal is in reasonably good condition. Typical records from the rabbits' cervical sympathetic and from various abdominal nerves are given in Fig. 1. The discharge consists of diphasic potential waves travelling centrifugally (as indicated by the direction of the first phase). The waves vary in size, but as a rule the majority of them do not vary much in contour or duration. The usual controls (killing the nerve, substituting a moist thread, etc.) show that the waves are due to nerve impulses and are not artefacts caused by movement or by potential changes in some other part of the animal.

Before discussing the grouping of the impulses and the frequency of the discharge under different conditions, we have to deal with the form of the individual waves and the nature of the fibres which produce them. Do they represent the activity of single fibres or of groups working more or less synchronously, and are they due to pre- or to post-ganglionic fibres or to both? Since the sympathetic fibres are too small to be examined individually, it is impossible to decide the number

of fibres contributing to any given wave, but the potential changes are certainly larger than we might expect from single fibres of the sympathetic system. Many of them are at least three or four times as large as those produced in a nerve of the same size by single motor or sensory fibres of the somatic system. This may be shown by placing a small

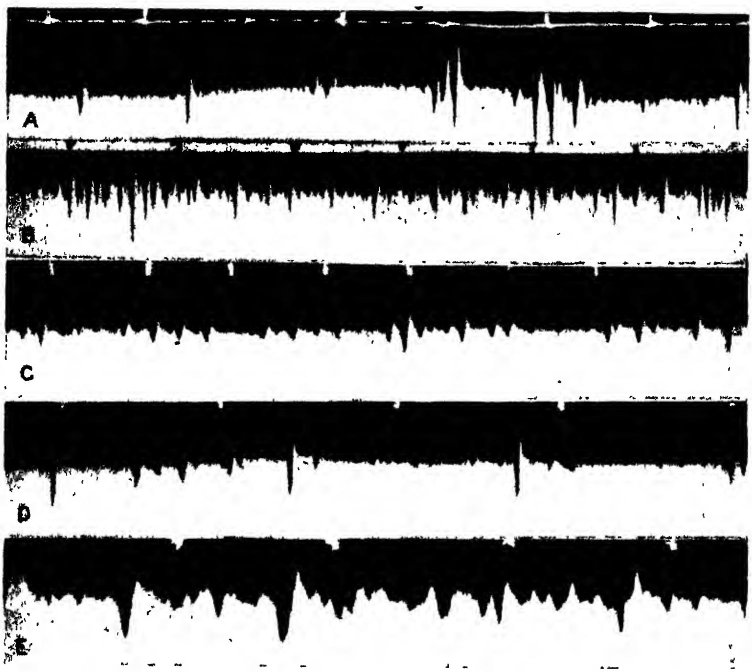

Fig. 1. Typical records of the persistent discharge in various sympathetic nerves. A Exp. 13. Rabbit, urethane. Nerve from the cœliac ganglion to the inferior vena cava. B. Exp. 23. Rabbit, urethane. Left cervical sympathetic. C. Exp. 19. Cat, decerebrate. Left hypogastric nerve. D. Exp. 2. Cat, urethane. Left pre-sacral nerve (from lumbar chain to inferior mesenteric ganglion). E. Exp. 3. Cat, decerebrate. Nerve from the inferior mesenteric ganglion to the gut. Time marker (at the top of each strip) in these and all low speed records gives intervals of 0·25 sec.

motor nerve (*e.g.* the top root of the phrenic) side by side with the cervical sympathetic on the electrodes and recording both somatic and sympathetic discharges simultaneously. The short circuiting by inactive tissue will be the same for both nerves, and it is found that the slow sympathetic waves are much larger than the rapid waves in the phrenic. As the sympathetic nerves do not give a greater potential change than the somatic when they are injured or stimulated electrically it is unlikely

that the potentials developed by each fibre are greater, but the general arrangement of the sympathetic system makes it quite likely that groups of fibres would be found working in very close connection, and a grouping of this kind would account for the large size of the waves.

It is probable, then, that the larger waves are due to groups of nerve fibres acting synchronously, but, if so, the different fibres in the group are often so well synchronized that they may be regarded as a single unit. This follows from the identical time relations of many of the waves: Fig. 1 shows that the interval between the two phases is fairly constant in any given record, and the uniform contour can be seen more clearly in Fig. 2 which gives several records made from the cat's hypogastric nerve with a high speed camera. It should be added, however, that besides the waves of constant form there are often some of more complex shape and longer time relations. One showing a double crest is given in Fig. 2 D. In two abdominal nerves all the waves have been very long and obviously complex, although the diphasic character is still retained (Fig. 1 E). Such waves are evidently due to volleys in a number of fibres discharging not quite simultaneously.

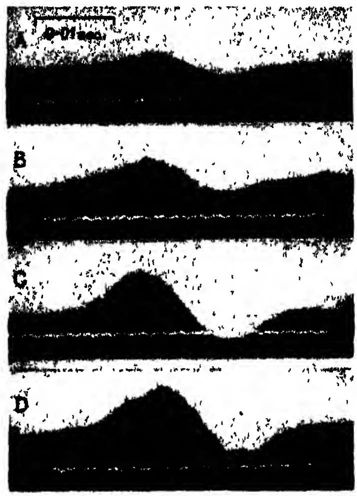

Fig. 2. Records of individual waves in hypogastric nerve discharge. A, B and C are examples of simple waves of different size. D is complex. Exp. 19. Cat, decerebrate. Left hypogastric nerve. 9 mm. between electrodes. Temp. 32° C. Time marker gives intervals of 0·0025 sec.

Rate of conduction.

When the discharge consists mainly of simple waves of constant form we may fairly assume that the potential change in each fibre has the same form, whatever may be the number contributing to the wave. As Bishop, Erlanger and Gasser [1926] have shown, it is difficult to secure monophasic recording by damaging the nerve when its time relations are very slow, but we can gain some idea of the rate of conduction by varying the distance between the electrodes. Fig. 3*a* gives three diphasic waves from the cat's hypogastric made with electrode separations of 5, 9 or 15 mm. Waves of approximately the same size have been chosen, but in each case the wave has the same time relations as the

others in the same record. By assuming that the potential change under each electrode has the form shown in Fig. 3*b* it is possible to reconstruct the three diphasic waves with fair accuracy. The rate of conduction then works out at 0·8 metre a sec. and this agrees very well with the rate of conduction of the potential wave produced by electric stimulation, for Fischer [1911] gives 0·7 metre a sec. for the splenic nerve of the ox and pig, and Erlanger and Gasser [1930] give 0·7 to 1·0 metre a sec. for the slow fibres in the white and grey rami (dog and cat). It agrees also with Dennig's measurements [1929] in which the latency of the end effect was recorded with stimuli at different points on the nerve, his

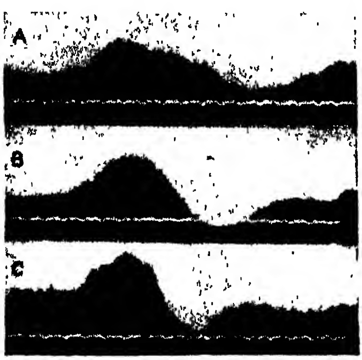

Fig. 3*a*.

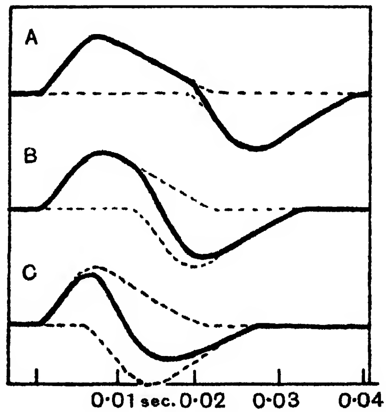

Fig. 3*b*.

Fig. 3*a*. Form of diphasic response with different electrode separations. Exp. 19. Cat, decerebrate. Left hypogastric nerve. Temp. 32° C. In A the distance between the electrodes was 15 mm., in B 9 mm. and in C 5 mm.

Fig. 3*b*. Reconstruction of potential change assuming a monophasic wave form as shown and a rate of conduction of 0·8 metre a sec.

figures being 0·8 metre a sec. for the nerve fibres to the sweat glands of the cat's foot and 0·71 metre for those to the nictitating membrane. The experiment shown in Fig. 3 was the only one in which we measured the rate of conduction, but the time relations of the diphasic waves in other experiments were always of the same order for a given electrode separation.

In their investigation of the potential waves produced by electric stimulation Erlanger and Gasser [1930] found that the grey ramus contains some fibres which conduct at 10–20 metres a sec. in addition to those conducting at 0·7–1 metre. The faster fibres, which form the B group in their classification, arise, or develop their characteristic rate,

in the sympathetic ganglia, since they are not found in the white rami or in the dorsal or ventral roots of the cord. We have not yet detected any potential waves which could be clearly ascribed to such fibres. Small and rapid fluctuations of potential have appeared occasionally, but we have not been able to decide whether they were true action potentials or merely interference effects caused by the overlapping of the slower waves. In some preparations of the cervical sympathetic there have been rapid ascending waves recurring in groups with the frequency of the heart beat, but further dissection has shown them to be due to fibres of the cardiac depressor running with the sympathetic trunk. It is usually possible to separate the strand giving the discharge of rapid impulses; it agrees in every way with the discharge of the cardiac depressor, and this is so characteristic that it can safely be used to identify the origin of the fibres.

PRE- AND POST-GANGLIONIC FIBRES.

According to Dennig and to Erlanger and Gasser the pre-ganglionic fibres conduct no faster than the post-ganglionic, but there might well be characteristic differences in the size of the individual waves or in their general arrangement. At present the only difference which we can be sure of is that the waves in a post-ganglionic discharge are on the whole larger than those in a pre-ganglionic. The origin of a given discharge can be decided by Langley's nicotine method, *i.e.* by painting ganglia with 0·5 p.c. nicotine solution or by injecting 10–30 mg. of nicotine into a vein. Painting the ganglion will break the connections in it between pre- and post-ganglionic fibres and injecting nicotine will break them throughout the body. By these methods we have found, as might be expected, that many of the potential waves appearing in the upper part of the cervical sympathetic are due to pre-ganglionic fibres, since they persist after a nicotine injection, and that those in the hypogastric nerve and other branches form the inferior mesenteric and cœliac ganglia are mostly post-ganglionic. The waves in the cervical sympathetic are fairly large and there is little to distinguish them from those in the other nerves, but in several experiments on the abdominal nerves where a ganglion has been painted, all the larger waves have dropped out leaving a succession of small waves of more uniform size. These may well be due to impulses in single pre-ganglionic fibres, the larger waves in the cervical sympathetic being due to a group of fibres acting in unison. It is, of course, to be expected that a post-ganglionic volley in a number of fibres acting as a unit would give a greater potential change than a

single pre-ganglionic impulse, for the bulk of the nerve fibres concerned must be much greater.

In two experiments on the cervical sympathetic, cut below the superior cervical ganglion, it was possible to detect waves passing both up and down the nerve. The ascending waves were smaller, and as they remained after a nicotine injection they were evidently pre-ganglionic. The descending waves, some of them very large, were abolished by nicotine, and presumably arose from a group of nerve cells embedded in the nerve trunk and sending post-ganglionic fibres down it. In one of the experiments the presence of the nerve cells was verified histologically. A record showing both ascending and descending waves is given in Fig. 4. Owing to the close spacing of the waves it is not possible to make out any definite relation between pre- and post-ganglionic

Fig. 4. Record from the cervical sympathetic showing ascending waves (first phase downward) and descending waves (first phase upward). The latter are post-ganglionic. The record also shows the respiratory grouping of the waves (cf. Fig 6). Exp. 20. Rabbit, urethane.

impulses, but it is evident that a preparation of this kind might be made to yield valuable information about the transference of activity from one neurone to another.

The function of the persistent discharge.

The discharges which we have recorded must be chiefly concerned in maintaining the tone of the blood vessels. It is known, for instance, that the cervical sympathetic exercises a persistent action on the blood vessels and on the plain muscle of the eye [Langley, 1900], but that its action on other structures (hairs and glands) is only occasional. The sympathetic nerves in the abdomen may exert a persistent inhibitory effect on the gut as well as a persistent vaso-constrictor effect on the blood vessels, but we have not succeeded in producing any clear modification of the discharge by stimulating the viscera, *e.g.* distending the bladder or pinching the gut. Indeed the only clear modifications have been produced by procedures which would be likely to affect the centres directly, such as the induction of asphyxia or the injection of nicotine,

and by drugs which would affect the blood vessels and so give rise to reflex changes in vaso-motor tone.

In the rabbit or cat an injection of adrenaline (0·5 to 1 mg.) into a vein is followed at once by a complete cessation of activity in the sympathetic nerves. The pause lasts as long as 5–10 minutes and there is then a gradual return of the discharge, beginning with occasional isolated waves at long intervals (Fig. 5). The injection of histamine (1–2 mg.) in the cat has been followed by a great increase in the discharge, and we have occasionally seen a definite increase after inhalation of amyl nitrite. These effects may be due in part to a direct action of the drugs on the sympathetic centres or on the blood vessels supplying them,

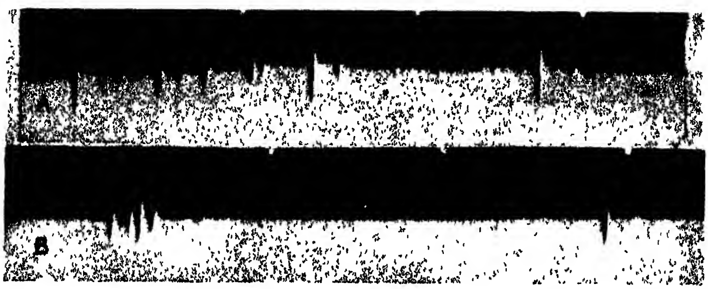

Fig. 5. Effect of adrenaline. Exp. 2. Cat, urethane. Pre-sacral nerve. A. Normal discharge. B. Return of the discharge after temporary abolition by an injection of 0·5 mg. adrenaline.

but the rise and fall of blood-pressure caused by the peripheral action of the drugs would be naturally followed by a reflex decrease or increase in the vaso-motor discharge. Moreover, adrenaline produces only a slight and evanescent change if the cardiac depressor nerves have been cut and the carotids tied above and below the sinus caroticus (two experiments). It is therefore a reasonable assumption that the discharge is influenced reflexly by the state of the vascular system, and that its function is mainly that of vaso-constriction. This conclusion lends an added interest to the fact discussed in the following section—namely, that the waves commonly occur in groups with the frequency of the heart beat or of respiration.

The grouping of the waves.

The respiratory grouping.

In the rabbit one of the most remarkable features of the persistent discharge is that the frequency of the waves often rises and falls in time with

the movements of respiration. The respiratory grouping was first noticed in the cervical sympathetic and we thought that it might be confined to this nerve, but it was then found that a rhythm just as well marked was usually present in the discharge of the abdominal nerves, *e.g.* those from the cœliac ganglion to the inferior vena cava. The respiratory rhythm has been definitely present in eight rabbits with vagi intact (three under chloralose and five under urethane) and absent initially in three (all under urethane). It has always been very well marked after section of the vagi (four experiments). Most of the experiments on cats were made before we had realized the importance of recording the respiration, but in the discharge of the abdominal nerves in decerebrate animals it has only once been possible to detect a respiratory grouping. The much slower rate of breathing may account for the difference, for in one cat under urethane with a respiratory rate of 30 a min. there is a well-marked grouping in the discharge of a nerve from the cœliac ganglion, and in another under chloroform the grouping is present in the hypogastric.

The effect is best shown in records made on a slowly moving surface. The characteristic appearance of such a record may be seen in Fig. 4, and others are given in Fig. 6. The tracing of respiration is made by a tambour leading from a 3-litre bottle which is connected to one branch of the tracheal cannula. The lever gives a downward movement on the record at the beginning of expiration; as the range of movement was very small when the other branch of the tracheal cannula was open to the air the records were usually made just after this was closed. The rate and depth of breathing were not affected for the first half-minute, and the use of the closed system gave a convenient means of testing the effect of increased CO_2, etc.

In the rabbit when the rhythm is present the discharge is at its maximum at the end of inspiration and the beginning of expiration. In animals with the vagi cut there is usually a definite pause during the latter part of expiration, but with the vagi intact there may be occasional waves throughout this period. When the respiration increases in depth and frequency as the animal breathes into the closed system the sympathetic discharge increases and the pauses may be obliterated, but the greatest activity always occurs in the same part of the respiratory cycle. When the tracheal tubes are clamped the same relation persists, the sympathetic discharge occurring at the height of each inspiratory spasm. In one decerebrate cat the discharge occurred in the hypogastric at the beginning of inspiration; in two other cats (anæsthetized) it persisted throughout inspiration.

An interaction between the respiratory and vaso-motor centres is known to occur under certain conditions, for the heart rate is often increased during inspiration by inhibition of vagal tone, and after curare the blood-pressure may continue to show waves with a respiratory

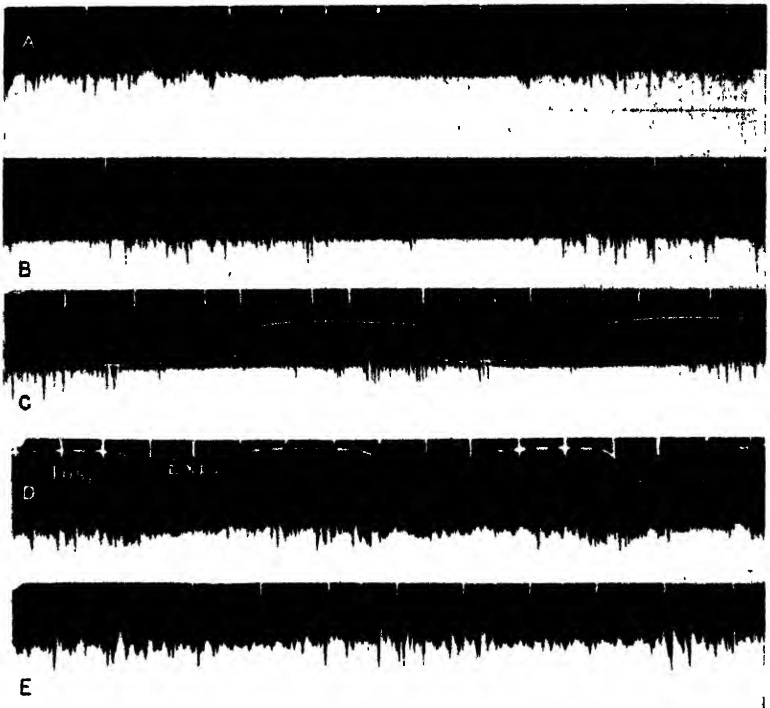

Fig. 6. Records from different nerves showing the grouping of the sympathetic discharge in phase with respiration. A. Exp. 11. Rabbit, chloralose. Right cervical sympathetic. B. Exp. 13. Rabbit, urethane. Vagi cut. Nerve from cœliac ganglion. Normal breathing. C. Exp. 13. Rabbit, urethane. Dyspnœa from breathing into closed system. D. Exp. 14. Rabbit, urethane. Left cervical sympathetic. E. Exp. 27. Cat, chloroform and ether. Left hypogastric. In the record of the breathing a downward movement denotes expiration.

rhythm although the chest is motionless[1]. It seemed worth considering, however, whether the grouping of the sympathetic discharge in the present experiments might not be due to some reflex or mechanical effect arising from the movements of the chest wall. Sensory impulses from

[1] Daly [1930] has repeated Fredericq's experiment in which the blood-pressure was recorded after the thorax and abdomen had been opened and the phrenic nerves cut. The pressure then falls during inspiration and rises during expiration. (Experiments on cats.)

the vagus could be ruled out, since the rhythm was most clearly marked when both vagi had been cut. To eliminate the effect of the lung movements one experiment was made in which the thorax was opened and the lungs were inflated by a pump. During the artificial respiration the discharge in the cervical sympathetic was irregular, but when the pump was stopped a definite rhythm appeared in time with the movements of the ribs. In this animal the vagi were intact, and the absence of the

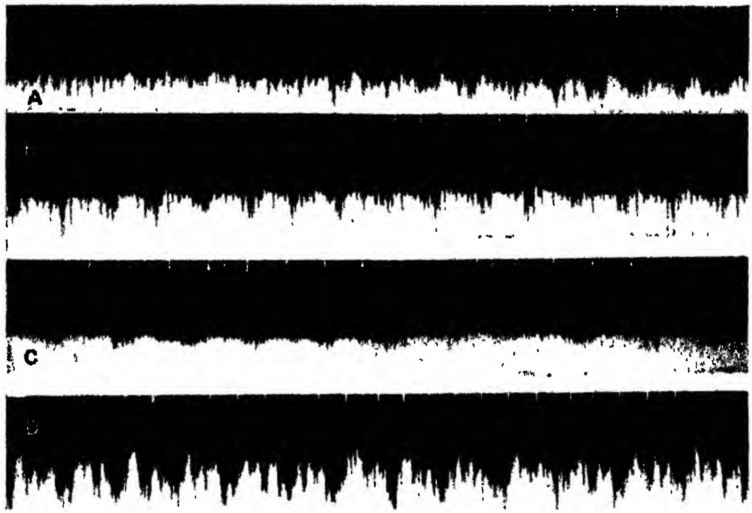

Fig. 7. Grouped discharges in the cervical sympathetic persisting after active respiratory movements have been paralysed by curare. Exp. 23. Rabbit, urethane. Vagi cut and carotids tied. Artificial ventilation. A. The rhythm is absent during over-ventilation, but returns as soon as the pump is stopped. B and C. The rhythm is independent of the frequency of the passive chest movements. In both records the groups occur at the rate of 71 a min. D. Asphyxia. The discharge is increased, but there is still some evidence of grouping. In the record of artificial respiration a downward movement denotes inflation of the chest.

rhythm during artificial ventilation may have been due to the sensory impulses reaching the respiratory centre at each inflation of the lungs and interfering with the proper rhythm of the centre. In three more experiments the preparation was made so as to allow a more complete isolation of the brain stem from sensory impulses. The vagi and cardiac depressors were cut and the carotids were tied, in one case both above and below the sinus caroticus. Enough curare was injected to stop all movement and the respiration was continued artificially. In all three experiments the sympathetic discharge occurred in well-marked groups

at intervals of 1–2 sec., *i.e.* at intervals corresponding to the respiratory rate before curare. Records are given in Fig. 7, and it will be seen that the rhythm of the discharge is independent of that of the artificial respiration. In fact an increase in the rate of ventilation causes a decrease in the rhythm and *vice versa*, though the change in rhythm does not often amount to more than 10 p.c. When ventilation is stopped the number of waves in each group increases and the rhythm quickens, though again the change is not very great. At a later stage, as the blood-pressure begins to rise, the intervals between the groups become filled up, but the rhythm can still be detected by ear, although in the records the waves are often too closely crowded to show it.

In these experiments the rhythm of the sympathetic discharge could not have been determined reflexly by the movements of the lungs or chest wall, but there was still a possibility of reflex control from the sensory effects which might result from each sympathetic outburst. To eliminate this one of the animals was given an injection of 30 mg. of nicotine to break the connection between pre- and post-ganglionic fibres. The rhythm remained as definite as before, though it was increased in rate by the injection. In this animal as the vagi were cut and the motor and sympathetic nerves paralysed, the rhythm could only be maintained reflexly through the action of the remaining parasympathetic nerves, and the likelihood of this must be very small.

It remains to prove that in the curarized animal the rhythm of the sympathetic discharge still keeps pace with that of the respiratory centre, for although the rate is altered by asphyxia or over-ventilation it is conceivable that the sympathetic centres are now working with an automatic rhythm of their own. The point can be decided by recording the impulses in the phrenic nerve as well as those in the cervical sympathetic. The uppermost root of the phrenic is easily accessible in the rabbit, and when cut distally it can be looped back so as to lie at the side of the cervical sympathetic on the recording electrodes (Fig. 8).

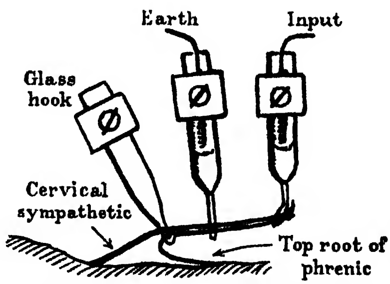

Fig. 8. Arrangement of electrodes for recording simultaneously from the cervical sympathetic and the highest root of the phrenic.

The motor impulses in the phrenic give brief action potentials which make a much greater noise in the loud speaker than the slow sympathetic waves, but the magnitude of the potential changes is less and the phrenic discharge may be almost invisible in a record

showing large sympathetic effects. To equalize the slow and fast waves one of the coupling condensers in the amplifier was made small enough to reduce the size of the slow waves without affecting the fast. A capacity of 0·006 mfd. was found by trial to give records in which both could be distinguished.

A record showing both the phrenic and sympathetic discharge in the curarized animal with vagi cut is given in Fig. 9, together with records of the two nerves separately, one taken immediately after the other.

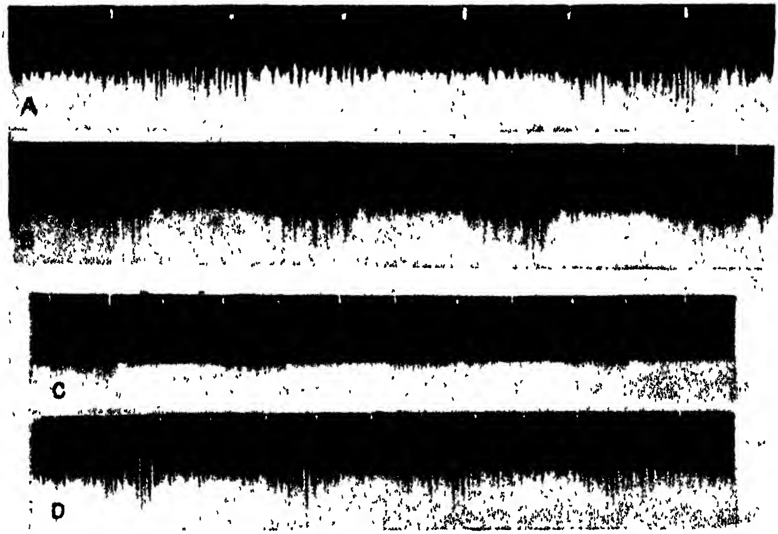

Fig. 9. Sympathetic and phrenic discharges in the curarized animal with artificial respiration. Exp. 23. Rabbit, urethane. Vagi cut and carotids tied. Sympathetic waves reduced in size by 0·006 mfd. Coupling condenser. A before and B shortly after an injection of 30 mg. of nicotine. The sympathetic discharge (slow waves) keeps in phase with the phrenic (rapid waves), though the phrenic outbursts are of shorter duration. The individual waves are scarcely visible in the record as reduced, but the pause between each combined outburst can be seen clearly. C. Record from the phrenic root alone. D. Record from the cervical sympathetic alone, taken shortly after C.

The phrenic discharge begins well before the main sympathetic outburst and ends well before the latter has begun to decline. The same relation between the two discharges was preserved throughout the experiment, though the rhythm was varied by under or over-ventilation, asphyxia, nicotine injection, etc., the only change being that in partial asphyxia the sympathetic discharge was more continuous whereas the phrenic still gave distinct outbursts with pauses between. It follows that in the absence of sensory impulses from the vagi the sympathetic centres in the

rabbit are directly stimulated by each period of activity in the respiratory centre. When the vagi are intact it is possible that the sensory discharge at inspiration also affects the sympathetic centres directly, though it seems more likely that the respiratory centre is still the controlling factor. But the occasional absence of the respiratory rhythm in rabbits and its more frequent absence in cats shows that the respiratory centre is not always in control, and when it is not it is interesting to find that the sympathetic discharge may now show a grouping corresponding to that of the heart beat.

The cardiac grouping.

A grouping of the sympathetic waves at a frequency equal to that of the heart beat has appeared in two rabbits and it was occasionally present in a third. A similar grouping appeared in three of the experiments on cats, though in two the heart beat was not recorded. In all these experiments there is a definite grouping of the waves, and the appearance of the cardiac rhythm is not due merely to movements of the base line caused by the pulsation of vessels transmitted mechanically to the nerve. Fig. 10 gives several discharges of this type together with records of the electrocardiogram made immediately afterwards. In the rabbits the rhythm was present initially with the animal under urethane and breathing quietly, and in all it was ultimately replaced by the usual respiratory rhythm. In one experiment section of both vagi brought about the change and in another an injection of adrenaline. In the third the cardiac grouping disappeared when the carotids were tied (the cardiac depressors having been cut previously). As it has appeared so seldom we cannot say whether it would invariably disappear when the sensory impulses from the depressors and sinus caroticus nerves are cut off, though it seems most likely that the grouping must be dependent on the rhythmic sensory outbursts reaching the brain stem from these nerves. It must be admitted that the close agreement with the cardiac rhythm might be merely a matter of chance, for the effect of varying the heart rate was not investigated; but although the regular appearance of single waves at intervals of 0·2–0·3 a sec. might have nothing to do with the heart beat, it is unlikely that all the waves in the discharge would keep so closely together unless they were controlled by some dominant rhythm.

The presence of these rhythms shows that the sympathetic centres are capable of fairly rapid fluctuations in activity, and that the conditions to which they are exposed are never likely to be steady. As long as the

respiratory centre is intact its activity will presumably form a background of waxing and waning excitation, even though its effect may be too slight to appear in the discharge, and the afferent nerves from the blood vessels will also contribute a fluctuating stimulus with the rhythm of the heart beat. In view of these disturbing and competing factors it

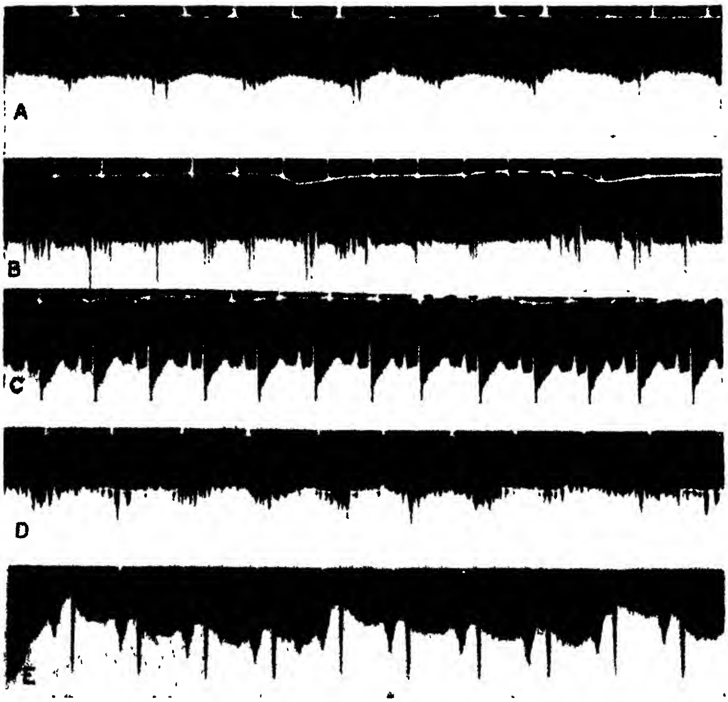

Fig. 10. Records showing the cardiac grouping of the sympathetic discharge. A. Exp. 16. Cat, urethane. Hypogastric nerve. B. Exp. 13. Rabbit, urethane. Nerve from cœliac ganglion. C. Exp. 13. Electrocardiogram made immediately afterwards. In this animal the respiratory grouping appeared after section of the vagi; it is shown in Fig. 6 B and C. D. Exp. 18. Rabbit, urethane. Cervical sympathetic. Dyspnœa from rebreathing. E. Exp. 18. Electrocardiogram made soon after. In this animal the respiratory grouping appeared after the discharge had been temporarily abolished by an adrenaline injection.

is easy to see why the sympathetic nerves never show a regular succession of waves comparable to the regular discharge of impulses from an end-organ or a motor neurone exposed to a steady stimulus. When neither cardiac nor respiratory rhythm can be detected the discharge is usually intermittent with pauses as long as half a second between some of the waves (cf. Fig. 1), though in asphyxia it becomes continuous.

Discussion.

The connection between the vaso-motor and respiratory centres.

The persistent discharges which we have recorded must be mainly vaso-constrictor in their effects. They are increased in asphyxia as the blood-pressure begins to rise and they are modified, as we should expect vaso-constrictor discharges to be modified, by the injection of drugs. The question then arises whether the grouping of the discharge in phase with respiration is of any practical value to the animal. Since the movements of the chest produce fluctuations of the blood-pressure by their mechanical effects on the vessels, it is possible that the fluctuating sympathetic discharge might tend to counteract these effects and to equalize the pressure at inspiration and expiration. In rabbits under the conditions of our experiments the maximum fall of blood-pressure was found to occur at the end of inspiration and the maximum rise at the end of expiration. The sympathetic discharge is at its height at the end of inspiration, and if its effect on the blood vessels were immediate it would counteract the fall of pressure. But it is unlikely that the mechanical effects would always tend to produce a fall of pressure at the moment when the sympathetic discharge tends to produce a rise. As Lewis [1908] has shown, the effect of the respiratory movements on the blood-pressure depends on a number of factors, and the phase relation may vary with the rate and depth of breathing, the muscles employed, etc. The sympathetic discharge seems to reach its maximum at a fixed period in the respiratory cycle, so that under some conditions it might accentuate the pressure changes instead of smoothing them out. It is also unlikely that each group of impulses would produce an immediate vaso-constriction, for with artificial stimulation of a sympathetic nerve there is a latency of a second or more before the blood vessels begin to contract. Indeed the vessels react so sluggishly that the degree of contraction is not likely to fluctuate at all when the impulse groups recur at intervals as short as 1 sec. In the curarized rabbits when the artificial respiration was stopped the blood-pressure as recorded by a mercury manometer rose smoothly in two experiments in spite of a definite respiratory grouping in the sympathetic discharge. In the third the blood-pressure appeared to rise in a series of steps, but unfortunately the rise was too great for the range of the recording system which was used in this experiment, and we cannot say where the steps appeared in relation to the outbursts in the nerve.

Since it seems improbable that the respiratory grouping is of much

value in preventing fluctuations of blood-pressure we must regard it as the natural consequence of the close connection between the vaso-motor and respiratory centres. The respiratory centre is the region most sensitive to changes in the blood, and its connection with the vaso-motor centre will enable the latter to react promptly to changes which are too small to affect its own less sensitive cells. In the goldfish brain stem Adrian and Buytendijk found that the potential gradients accompanying each phase of activity in the respiratory centre were readily detected in any part of the medulla. It is at least conceivable that these potential changes are enough in themselves to modify the activity of the vaso-motor centre without direct nervous connection, though it must be admitted that the effect is to some extent selective, for it is probable that the vagal centre is inhibited during the period in which the sympathetic centre is excited.

Comparison of sympathetic and somatic discharge.

If we compare the tonic sympathetic discharge with the discharge in a motor nerve maintaining the contraction of a skeletal muscle, the main difference is found in the much smaller number of potential waves in the sympathetic and in their greater size. If we are right in supposing that the larger waves are due to a number of fibres acting synchronously, the difference expresses the fact that the sympathetic system is arranged for the wide distribution of an activity arising in relatively few neurones in the cord. It is well known that the number of post-ganglionic fibres greatly exceeds that of the pre-ganglionic—for a particular case Billingsley and Ransom [1918] give the ratio as 32 to 1—and that the stimulation of a few pre-ganglionic fibres may produce widespread effects. If the ganglion acts merely as a region where one path branches into many, the post-ganglionic discharge would naturally consist of synchronous impulses in large groups of fibres. The effectors supplied by the sympathetic system react so sluggishly that it can make little difference whether the impulses are discharged by synchronous volleys widely spaced or by independent fire in each nerve fibre, whereas in the somatic nerves it can make all the difference between a steady contraction or a tremor, and independent firing is the rule except at high frequencies.

We are not in a position to say whether the pre-ganglionic fibres usually act independently or in groups, or whether a single pre-ganglionic impulse or volley ever sets up a succession of post-ganglionic volleys. It is clear that this does not always happen, for a post-ganglionic dis-

charge, when cut down almost to vanishing point by an injection of adrenaline, usually consists of isolated waves separated by intervals of a second or more; each of the waves may appear at about the same time in the respiratory cycle, and it is therefore unlikely that any of them can be due to an after-discharge from the ganglion cells. On the other hand the ganglion cell, or some part of the neurone other than the axon, can be made to give a repeated discharge at a fairly high frequency. When a ganglion is painted with 1 p.c. nicotine there are often sudden outbursts of waves in a regular series which rises in frequency to about 100 a sec. and falls more slowly. These outbursts are comparable to those produced by injury in certain mammalian nerve fibres and in the nerve ganglia of insects, though their maximum frequency is lower (judged by ear and by observation with the revolving mirror). In one experiment on the hypogastric outbursts of this kind were observed for a short time immediately after the preparation was set up. They arose from a point in the nerve between the two electrodes, and on examination afterwards the nerve was found to contain a small group of nerve cells in this region. Apart from this instance, where the nerve cell outbursts were probably due to injury in dissection, and from another where the origin of the discharge was more uncertain, we have found no evidence of spontaneous activity in ganglion cells.

Many points have been left unsettled, but so far it may be said that although some of the features of the sympathetic discharge have been unexpected, we have found nothing to conflict with what is known of the reactions of nerve cells and fibres in general or of the structure and functions of the sympathetic system.

Summary.

The persistent discharges which occur in mammalian sympathetic nerves have been investigated in the cat and rabbit by amplifying the potential waves in the nerve and recording them photographically. The waves are slow diphasic potential changes conducted at a rate of about 0·8 metre a sec. (one experiment). The larger waves are mostly post-ganglionic and they are probably due to volleys of impulses occurring in groups of sympathetic fibres, since they are several times as great as the action potentials produced in a nerve of the same size by single motor or sensory fibres. Some of the waves are obviously complex and due to volleys which are not quite simultaneous.

The discharges which have been recorded are mainly concerned with

vaso-constriction. In the majority of the experiments the waves tend to occur in groups with the frequency of the heart beat or of respiration. The respiratory grouping is due to a direct action of the respiratory centre on the vaso-motor centre, for it persists in the curarized animal after artificial respiration has been stopped and the outbursts in the sympathetic are still in phase with the motor discharges in the phrenic. When the respiratory grouping occurs the maximum discharge coincides with inspiration.

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THE SIGNIFICANCE OF THE PITUITARY IN PARTURITION.

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It is now known that extracts of the posterior lobe of the pituitary gland have a specific stimulating action on the uterus probably greater than that of any other known substance, and such extracts are used by physicians to promote labour pains, even in preference to ergot and other drugs. As pituitrin is believed to be present as a normal constituent of blood the question arises as to how far this substance is responsible for the normal production of labour.

It has been suggested by Dixon and Marshall [1924] that pituitrin is secreted at the end of pregnancy under the stimulus of certain substances produced at this time which are stated to have their origin chiefly in the ovary. The increase in the pituitrin content of the body fluids during the last days of pregnancy would thus stimulate the uterus and lead to parturition. Other investigators, Bourne and Burn [1928] and Parkes [1930], however, have denied the increase in the secretion of pituitary at the end of pregnancy and suggested that some "sexual hormone," circulating in the blood during pregnancy in larger amounts than normal, sensitizes the uterus to the action of pituitrin.

The view that the posterior lobe of the hypophysis produces at the end of pregnancy more pituitrin than is normally present was put forward by Dixon and Marshall [1924] as a result of their experiments with dogs. They found that cerebrospinal fluid obtained by puncture of the cisterna cerebello-medullaris in dogs into which extracts from the ovaries of pregnant pigs and rabbits had been injected, had a greater stimulant action on the isolated uterus than the cerebrospinal fluid of untreated animals. The ovarian extracts were effective only when prepared during œstrus, or from pregnant animals shortly before delivery. They believed that the increase in oxytocic activity of the cerebrospinal fluid was due to an increased pituitrin content. It is now well recognized that pituitrin after secretion into the cerebrospinal fluid is rapidly

absorbed into the circulation and that little or none ever reaches the lumbar region of the cord [McLean, 1928; van Dyke, Bailey and Bucy, 1929]. The observations of van Dyke and Kraft [1927] and the comments made on them by Whitehead and Huddleston [1931] therefore need not be considered.

It is possible to use quite a different method to obtain some knowledge of the secretion of the posterior lobe of the hypophysis during pregnancy. It is known that adrenaline relaxes the virgin uterus of most laboratory animals, but that the uterus of a pregnant animal behaves differently according to the kind of animal. Thus the pregnant cat's uterus is stimulated by adrenaline, whereas the pregnant uterus of guinea-pigs and rats is said to be relaxed in the same way as that of the virgin animal [Gunn and Gunn, 1914; Cow, 1919]. Trendelenburg [1923], summarizing the results of other investigators, states that most observers found a relaxation of the pregnant uterus of the guinea-pig after administration of adrenaline, but that in some cases stimulation was observed. We show in this paper that both findings are correct under certain well-defined conditions.

Cow, working in this laboratory, found in experiments on guinea-pigs and virgin cats that previous treatment of the isolated uterus, or the intact living animal, with pituitrin reverses the effect of adrenaline so that it produces a contraction. He concluded from these investigations that the amount of pituitrin available in the body is a factor which decides the nature of the reaction of the uterus to adrenaline. In view of Dixon and Marshall's theory it is natural to suggest that the increase in the pituitrin content of the body fluids in pregnancy is the cause of the altered response of the uterus during this period.

As a satisfactory method of determining the pituitrin content in the blood is not available at the present, we have tried to use Cow's observations (reversal of the adrenaline response of the guinea-pig's uterus by preliminary treatment with pituitrin) as the foundation of a method of detecting changes in the activity of the posterior lobe of the pituitary during pregnancy. The effect of adrenaline on the uterus of guinea-pigs at different stages of the œstrous cycle and of pregnancy has therefore been examined.

The work of Bourne and Burn has raised the question of the extent to which the onset of labour is determined by a sensitization of the uterus by œstrin rather than by an increase in the amount of pituitrin in the blood.

Our study of the sensitization led us to repeat these experiments of

Bourne and Burn and also to investigate the influence of a change in the K/Ca ratio and of adding anterior pituitary hormone to the isolated uterus; since alteration in both these factors is regularly found in pregnancy.

METHODS.

Guinea-pigs were used; the animals were killed by a blow on the head, the vessels in the neck were severed, and the animals rapidly bled. One horn of the isolated uterus, or, in the case of a pregnant animal, a longitudinal strip, was suspended in a bath containing 100 c.c. of fluid, which was kept at 38° C. The bath was filled either with Tyrode solution or with that used by Burn and Dale [1922]; in one section of our work solutions with varied calcium and potassium content were used. The composition of the solution is therefore mentioned in the description of every experiment quoted. The bath was kept oxygenated by a constant stream of oxygen. After the uterus had been suspended in the warm solution for a sufficient time (10–15 min.) to allow the tonus to become steady, a normal record was taken for another 10 min. The drugs were injected rapidly into the bath fluid by means of a syringe.

It is well known that the contraction produced by a given dose of pituitary extract tends to become more marked each time that the drug is added. With some uteri, however, especially infantile ones, a state is reached after three to five additions, when a dose of pituitrin gives the same submaximal response many times. The contractile response of other uteri increases steadily with each addition of pituitary, until a stage is reached when even smaller doses than those originally employed produce maximal contractions. (All-or-none uteri.) It is very important to eliminate these "all-or-none uteri" in an investigation concerned with substances of which the sensitizing action for pituitrin is being determined. In our experiments, therefore, only those uteri were employed which gave the same response three times at least.

The stage of the œstrous cycle of all the animals used was examined by Allan and Doisy's method of vaginal smears.

EXPERIMENTAL.

The action of adrenaline on the uterus of the guinea-pig during different stages of the œstrous cycle and of pregnancy.

The experimental observations of Dixon and Marshall [1924] point to the fact that the response of the tissues in the body of the

pregnant animal to stimuli are not the same throughout pregnancy. For example, differences certainly exist between the last days of pregnancy shortly before delivery and the earlier stages of pregnancy, and between the œstrous and the diœstrous stage. We have therefore investigated the effect of adrenaline on the virgin and pregnant uterus of the guinea-pig during the different stages of the œstrous cycle and of pregnancy. Table I given below shows the results of the experiments.

TABLE I. The effect of adrenaline on the uterus of virgin and pregnant guinea-pigs at different stages of the œstrous cycle and of pregnancy (+contraction; −relaxation).

Virgin			Pregnant	
Infantile	Diœstrous	œstrous	Commencement of pregnancy	Shortly before delivery
−	−	+	−	+

It will be seen from this that the infantile uterus and that of the sexually mature animal in the diœstrous period were relaxed by adrenaline, and this is true also for the pregnant uterus during the greater part of pregnancy (Fig. 1). The non-pregnant uterus of the animal in œstrus and the pregnant uterus just before delivery are stimulated by adrenaline (Fig. 2). The result of our experiments on the œstrous uterus confirms the observations of Kochmann and Seel [1929].

The result of our experiments with adrenaline, in which the pregnant uterus shortly before delivery gives a reverse action to adrenaline (Table I), suggests that this reversal in the effect of adrenaline is caused by an increase in the output of pituitrin, as Cow was able to show that the relaxing effect of adrenaline on the guinea-pig uterus is reversed to stimulation by previous treatment with pituitrin.

The action of œstrin on the isolated uterus.

Bourne and Burn [1928] do not think that the presence of an increased secretion of pituitrin at the end of pregnancy is necessary for the physiological production of parturition. From their experiments on the sensitizing effect of the ovarian hormone (œstrin) on the isolated uterus to the pituitary stimulus, they concluded that œstrin, which is produced in large quantities during pregnancy, is the important agent, and that at the end of pregnancy a critical moment occurs when the uterus becomes so sensitive that it responds to the normal amount of pituitrin present and that this is followed by birth.

We have endeavoured to verify the experiments of Bourne and Burn, and like them found at first that the effect of pituitrin on the

isolated uterus is definitely strengthened by an œstrin preparation given previously (Fig. 3). (Messrs Parke, Davis' preparation "estrogen" was

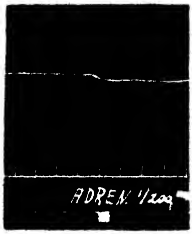

Fig. 1.

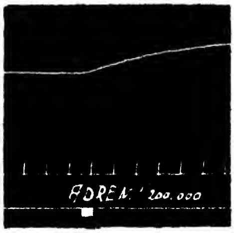

Fig. 2.

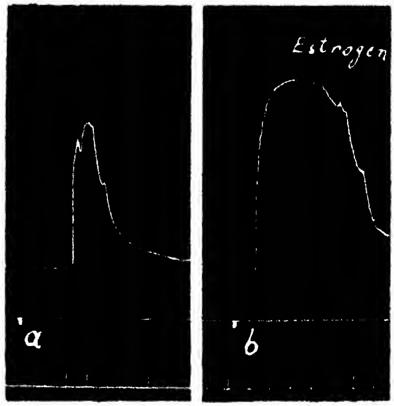

Fig. 3.

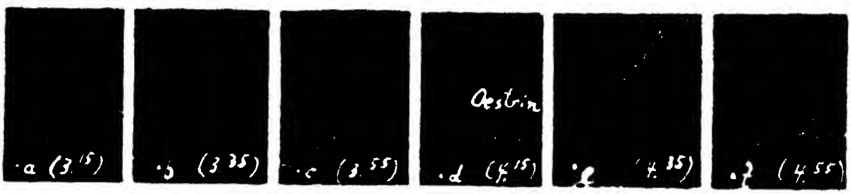

Fig. 4.

Fig. 1. Guinea-pig's uterus. Early pregnancy. 100 c.c. bath. Relaxation by adrenaline (1·0 c.c. Adr. 1/2000). Time = minutes.

Fig. 2. Guinea-pig's uterus. Late pregnancy. 100 c.c. bath. Stimulation by adrenaline (1·0 c.c. Adr. 1/2000). Time = minutes.

Fig. 3. Guinea-pig's uterus, infantile. 100 c.c. bath. Sensitization to pituitrin by estrogen (an impure œstrin preparation). (*a*) 0·5 c.c. pituitrin 1/500; (*b*) 0·5 c.c. pituitrin 1/500 (10 min. after 0·5 unit estrogen was added). Time = minutes.

Fig. 4. Guinea-pig's uterus, infantile. 15 c.c. bath. No sensitization to pitocin by crystalline œstrin. (*a*) 0·2 c.c. pitocin 1/3200 (7 min. after 0·2 c.c. control solution was added); (*b*) and (*c*) the same as (*a*); (*d*) 0·2 c.c. pitocin 1/3200 (7 min. after 0·02 mg. crystalline œstrin in 0·2 c.c. control solution was added); (*e*) 0·2 c.c. pitocin 1/3200 (7 min. after 0·75 c.c. control solution was added); (*f*) 0·2 c.c. pitocin 1/3200 (7½ min. after 0·2 c.c. control solution was added). Time: 1 min. = 3 mm.

used.) Fröhlich and Paschkis [1926] have pointed out, however, that very small quantities of proteins produce marked sensitization of the uterus to pituitrin, and we have therefore made experiments to

determine whether the sensitization by estrogen, which contains protein, is due to the ovarian hormone or is only an effect of the protein; it may be mentioned that Bourne and Burn state that the preparation examined by them was not protein-free. We therefore repeated the experiments with a preparation (progynon) which is entirely protein-free. This preparation had no stimulating action on the isolated uterus in doses up to 100 mouse units. We never found the slightest sensitization to pituitrin using this pure preparation in amounts a hundred times greater than those employed by Bourne and Burn. Therefore we are forced to the conclusion that the positive results obtained by Bourne and Burn as well as our own results with impure œstrin (estrogen) should not be regarded as specific for œstrin, but are probably due to the protein content of the preparations.

This view was confirmed in an experiment with crystalline œstrin, kindly supplied by Dr Marrian (Fig. 4). A solution was prepared in distilled water, to which 5 p.c. alcohol had been added (control solution), the strength being 1 c.c. = 0·1 mg. œstrin.

In the control experiments equal quantities of solvent were invariably added to the bath (Fig. 4*a*, *b*, *c*). The need for careful observation of this precaution is demonstrated in tracing Fig. 4*e*. In experiments Fig. 4*a*, *b*, *c*, 0·2 c.c. of control solution was added to the bath, which contained 15 c.c. of Ringer's solution. In experiment Fig. 4*d* the addition of 0·75 c.c. of control solution was sufficient to cause a dilution sensitization [Dale, 1913] to subsequently administered pitocin. This dilution sensitization is reversible, since repetition of the experiment with undiluted fluid and the same dose of pitocin produces the normal response (Fig. 4*f*).

No such sensitization was produced by even 0·02 mg. of œstrin, provided it was not dissolved in a greater quantity of control solution than 0·2 c.c. (Fig. 4*d*).

The sensitizing action of anterior pituitary hormone (prolan) for pituitrin.

Having investigated the effect of œstrin on the pituitrin response of the uterus we endeavoured to do the same for the anterior pituitary hormone, which, as is well known, is also present in increased quantity in the pregnant animal. For these experiments we used the preparations præhormon (Promonta) and prolan (I.G.), both prepared from the urine during pregnancy, protein-free and shown to be active (Zondek-Ascheim test). These preparations produce sometimes a slight contraction of the uterus.

It will be shown subsequently that an increase in the potassium content of the bath fluid also sensitizes the uterus to pituitrin. It is therefore important to know whether our results might be due to the potassium content of prolan.

To produce this potassium action an addition of at least 6 mg. p.c. must be made. In most experiments only 25 units of prolan were added

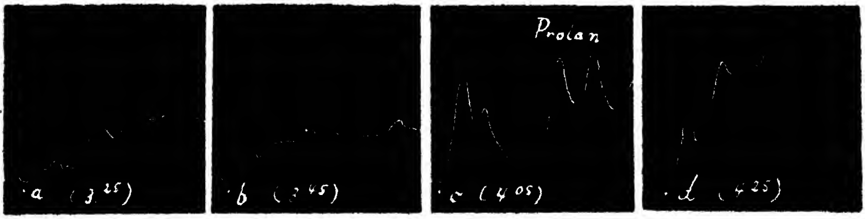

Fig. 5. Guinea-pig's uterus, infantile. 100 c.c. bath. Sensitization to pitocin by prolan (ant. pituitary hormone). (*a*) 0·2 c.c. pitocin 1/600 (5 min. after 0·5 c.c. dist. water was added); (*b*) the same as (*a*); (*c*) 0·2 c.c. pitocin 1/600 (5 min. after 0·5 c.c. prolan = 25 rat units was added); (*d*) 0·2 c.c. pitocin 1/600 (5 min. after 0·5 c.c. dist. water was added). Time: 1 min. = 3 mm.

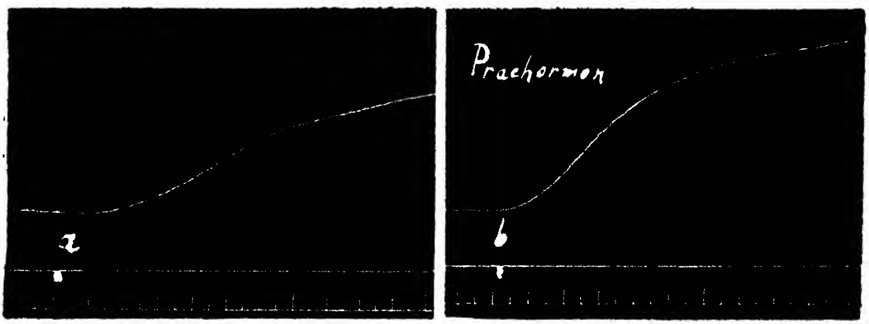

Fig. 6. Guinea-pig's uterus, pregnant. 100 c.c. bath. Sensitization to pituitrin by præhormon (ant. pituitary hormone). (*a*) 0·5 c.c. pituitrin 1/500 (10 min. after 1·0 c.c. dist. water was added); (*b*) 0·5 c.c. pituitrin 1/500 (10 min. after 1·0 c.c. præhormon = 50 rat units was added). Time = half minutes.

to 100 c.c. of bath fluid, and since the total ash content of these is only 0·3 mg. it is clear that the potassium content cannot be sufficiently great to affect our results.

Initial treatment of the isolated uterus with prolan or præhormon increases the response to pituitrin. This can be observed not only in infantile and diœstrous uteri (Fig. 5) but also in the pregnant uterus (Fig. 6). The doses used were 10–50 rat units of either prolan or præ-

hormon. It has been found that the sensitizing effect of both of these commercial preparations (prolan and præhormon) does not disappear even after several changes of the fluid in which the uterus is suspended (Fig. 5*d*).

The results of these experiments suggest sensitization of the uterus to pituitrin by the action of the anterior hypophysis hormone.

The influence of calcium and potassium on the pituitrin response of the isolated uterus.

It appears from the literature that a fall in serum calcium during pregnancy is the rule, particularly in the last months at a time when the mother needs at least twice her normal supply of calcium [Hoyle, 1930]: decrease in serum calcium and changes in the K/Ca quotient have been frequently reported [Damble, 1930; Hellmuth and Timpe, 1930; Frei and Emmerson, 1930]. This fact and the knowledge of the importance of correct ionic concentration for the activity of all organs innervated by the autonomic nerve system induced us to investigate the effect of pituitrin on the isolated uterus with varied concentrations of calcium and potassium.

Infantile and dicestrous animals only were used. In these experiments sometimes the standard fluid was changed for one with altered potassium and calcium content, the temperature remaining constant. In other cases the KCl and $CaCl_2$ dissolved in ½ c.c. of distilled water was added directly to the standard fluid.

Four series of experiments have been performed under the conditions given in Table II.

TABLE II.

Standard solution used	K/Ca ratio of standard solution	Changes of K/Ca ratio made in the experiments
(1) Tyrode	1/1	(*a*) − 25 p.c. $CaCl_2$ (*b*) − 50 p.c. $CaCl_2$
(2) Tyrode	1/1	(*a*) + 25 p.c. KCl (*b*) + 50 p.c. KCl (*c*) + 100 p.c. KCl
(3) Tyrode	1/1	(*a*) + 50 p.c. KCl and + 50 p.c. $CaCl_2$
(4) Burn-Dale	1·7/1	(*a*) − 25 p.c. $CaCl_2$ and − 50 p.c. $CaCl_2$

The isolated uterus of a virgin guinea-pig in Tyrode solution, which contains equal amounts of potassium chloride and calcium chloride, shows in most cases no spontaneous movements. If the uterus is placed in the solution used by Burn and Dale [1922], which contains a double amount

of potassium chloride, corresponding roughly to the amounts present in the blood, it is frequently found that the uterus shows spontaneous movements. The relatively high potassium content favours the spontaneous movements; this is in harmony with the investigations of Knaus and Clark [1925] and others, and from a large number of experiments we can confirm it.

Our experiments with the isolated uterus show that either an increase of potassium or a decrease of calcium in the solution, *i.e.* a relative predominance of potassium, leads to supersensitiveness to pituitrin. A decrease of calcium by 25 p.c. leads to no decided changes in the uterine contractions, but it renders the uterus more sensitive to pituitrin. This is demonstrated in Fig. 7 by an increase in the number of rhythmical

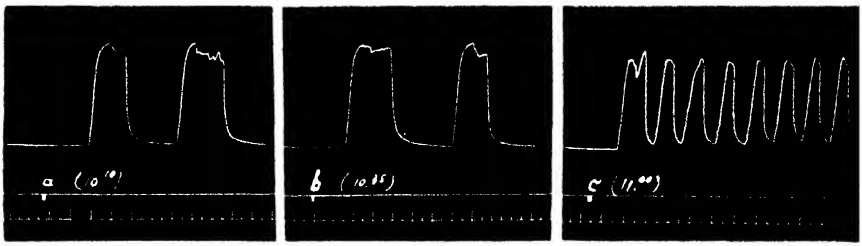

Fig. 7. Guinea-pig's uterus (dioestrous). 100 c.c. bath. Sensitization to pituitrin by potassium. The uterus is suspended in Burn-Dale solution. (*a*) 0·5 c.c. pituitrin 1/500; (*b*) 0·5 c.c. pituitrin 1/500; (*c*) 10 min. before (*c*) the Burn-Dale solution was exchanged for one containing 50 p.c. less calcium. Then 0·5 c.c. pituitrin 1/500 was again added. Time = half minutes.

contractions per minute, produced by a dose of pituitrin. In other experiments the sensitization to pituitrin was shown to be present by the increased tonus of the uterus (Fig. 8).

Experiments were performed under the conditions set out in Table II (3), which showed that an increase of potassium and calcium in the same proportion in the fluid does not alter the action of pituitrin. It is, therefore, the change of the K/Ca ratio, not the absolute amounts of K or Ca, which influence the pituitary response.

We desire to point to the parallel which exists between these experiments and the altered K/Ca quotient in the blood and the uterus of pregnancy [Kochmann and Krüger, 1926], and think that the relative preponderance of potassium, which *in vitro* produces decided sensitization of the uterus to pituitrin, might also have some significance *in vivo* in the process of labour.

We do not, however, believe this to be an important factor, as a simple change in the diet of the mother changes the K/Ca equilibrium to the normal. But it may well be possible that a large surplus of calcium in the body may influence the time of delivery. Nahmmacher [1930] has shown that pregnant women kept on a diet rich in vitamin D (vigantol) deliver 8 to 10 days later than the controls. This would be readily explained by the fact that vitamin D considerably increases the calcium absorption from the alimentary canal.

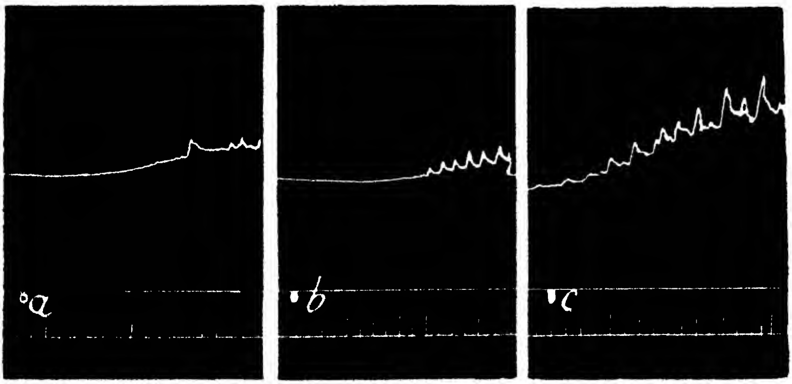

Fig. 8. Guinea-pig's uterus, infantile. 100 c.c. bath. Sensitization to pituitrin by potassium. The uterus is suspended in Burn-Dale solution. (a) 0·5 c.c. pituitrin 1/500; (b) 0·5 c.c. pituitrin 1/500; (c) 10 min. before (c) the Burn-Dale solution was exchanged for one containing 50 p.c. less calcium. Then 0·5 c.c. pituitrin 1/500 was again added. Time = half minutes.

Discussion.

The evidence that the oxytocic principle of the pituitary is of importance for the physiology of parturition seems conclusive. The objection has been raised, however, that hypophysectomized animals deliver normally. This objection seems hardly justified, since the loss of pituitrin caused by hypophysectomy is quickly amended by hypertrophy of the neighbouring tissues [Trendelenburg, 1927; Sato, 1928; Geesink and Koester, 1929].

It was found by Dixon and Marshall that an ovarian extract prepared from animals shortly before delivery produced an increase in the pituitrin content of the cerebrospinal fluid when given to non-pregnant animals. They suggested that this would be consistent with an increased secretion of pituitrin at the end of pregnancy.

In further support of this is the observation of Mayer [1924] that

human cerebrospinal fluid taken during normal delivery promotes labour in cases where parturition is delayed. Cerebrospinal fluid from non-pregnant women had no such property.

The experiments of Cow [1919] on the virgin guinea-pig uterus in which by pituitrin treatment he was able to change the inhibiting action of adrenaline into a motor effect is another indication of the probability of the Dixon and Marshall theory, since we were able to show that adrenaline excites the uterus of guinea-pigs only at the end of pregnancy. It is at the end of pregnancy that the amount of pituitrin in the blood is thought to be materially increased.

Blau and Hancher [1926] have raised doubts as to the significance of the results obtained by Dixon and Marshall because they state that the rise of the pituitrin secretion by ovarian extracts is not specific, as they obtained also with other tissue extracts an increase in the oxytocic activity of the cerebrospinal fluid. We think their objection is not justified, because the investigation of Dixon and Marshall only deals with a comparison between the conditions of the non-pregnant and those of the pregnant organism, therefore the "specific action" of the ovarian extracts used by them in the defined conditions is clearly valid.

The results of our experiments described in this paper indicate that not only an increase in the secretion of pituitrin, but also an increased sensitivity of the uterus to pituitrin probably occurs at the end of pregnancy and may be an important factor in determining the onset and progress of parturition. Our experiments suggest that this sensitization is produced by:

(1) *The hormone of the anterior pituitary.* We have found that initial treatment with commercial preparations giving the Zondek-Ascheim test (prolan and præhormon) increase the response of the isolated uterus to pituitrin.

In the case of œstrin we were fortunate in being able to use the crystalline and presumably pure substance. We wish to indicate, however, that in the absence of a pure anterior pituitary preparation we are unable to demonstrate with certainty that the hormone is indeed the active principle.

Since, however, both commercial preparations used were protein-free and it was shown that their potassium contents were negligible, it seems highly probable that the sensitization of the uterus is due to the hormone itself. Œstrin, on the other hand, has neither a stimulating nor a sensitizing action on the isolated uterus; we believe that the sensitizing effect

formerly ascribed to this substance is most probably due to impurities, as we did not get any sensitizing effects with crystalline œstrin.

(2) *Changes in the K/Ca ratio of the fluid in which the uterus is immersed.* We were able to show that a preponderance of potassium in the bath fluid distinctly sensitizes the isolated uterus to pituitrin. The K/Ca ratio of the blood alters during pregnancy, potassium becoming predominant. Of greater importance still seem the changes found in the substance of the uterus itself. Kochmann and Krüger, analysing normal and puerperal human uteri, found a decided change of the K/Ca ratio during pregnancy, the potassium content had increased, the calcium content diminished. It is possible that a similar significance may be attributed to the findings *in vivo* and *in vitro*.

The question why the increased production of the hormone of the anterior lobe and the alteration in the K/Ca ratio does not induce labour before the normal end of pregnancy probably finds its answer in the work of Knaus. The results of his experiments on the sterilized uterus horn of pregnant rabbits suggest that the uterus during the first half of pregnancy is insensitive to pituitrin by the action of the persistent corpora lutea of gestation. During the second half of pregnancy, when the corpora lutea are in regression, the uterus regains slowly its sensitivity to pituitrin. Just before parturition occurs a very rigid increase in the sensitivity of the uterus.

Our findings suggest that at this time when by degeneration of the corpora lutea their inhibiting action disappears, labour is induced by the stimulus of a comparatively small increase of pituitrin on a uterus sensitized to its action by a combination of factors such as the increase of anterior pituitary and the alteration in the K/Ca ratio.

Summary.

The significance of the posterior hypophysis hormone in parturition is discussed with respect to the following findings:

1. Adrenaline relaxes the uterus of the guinea-pig during the greater part of pregnancy and stimulates it shortly before delivery.

2. Protein-free œstrin (progynon) and crystallized œstrin have no sensitizing action for pituitrin on the isolated uterus.

3. Protein-free preparations of the anterior hypophysis hormone prepared from urine (prolan, præhormon) sensitize the uterus of infantile and pregnant guinea-pigs to pituitrin.

4. Changes of the K/Ca ratio of the bath fluid sensitizes the uterus to pituitrin, if potassium is predominant.

We are indebted to the late Prof. W. E. Dixon for help in many ways.

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NUCLEIC ACID DERIVATIVES AND THE HEART BEAT.

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IN a previous paper in which the physiological reactions of various nucleic acid derivatives were compared [Bennet and Drury, 1931], it was stated that the perfused rabbit's heart was improved, as a preparation, when adenosine or adenylic acid was added to the perfusate. Drury and Szent-Györgyi [1929] had found that adenosine did not influence the mechanogram of the heart in the intact dog, and Wedd [1931] obtained the same result for the perfused rabbit's heart. On the other hand Rothman [1930] and Lindner and Rigler [1931] found that both adenylic acid and adenosine strengthen the heart beat.

Both adenosine and adenylic acid increase the flow through the heart [Wedd, 1931], and it is reasonable to conclude that this will, under certain circumstances, be accompanied by an increased amplitude of contraction. For this reason Bennet and Drury [1931] made no observations upon the mechanogram as they felt they could not distinguish between the effects due to the greater flow of perfusate through the heart and those associated with a specific action of the substances upon the musculature. More recently the general physiological properties of guanylic acid have been under investigation, and as this substance decreases the flow through the perfused rabbit's heart, observations have been made upon the mechanogram, and the results allow a much more definite idea to be formed of the manner in which nucleic acid derivatives influence the heart beat.

The rabbits' hearts have been perfused through a cannula inserted into the aorta in the usual manner, Locke-Ringer solution at a pressure of 30–40 cm. being used. The perfusate has been oxygenated by bubbling oxygen through it. In certain experiments the animal has been bled, under ether anæsthesia, prior to removing the heart, and after defibrination the blood has been added to the Ringer solution. The whole preparation has been housed in a moist warm chamber at 37° C., so that

[1] Working on behalf of the Medical Research Council.

the temperature of the heart remains constant throughout the observations. The coronary outflow has been recorded by collecting the fluid leaving the heart in a tipping bucket, while the substances have been injected into the tubing carrying the perfusate to the heart. The injections, usually 1 c.c., have been made slowly, and have been brought if necessary to a *p*H of 7·4–7·6 by the addition of sodium bicarbonate. The mechanogram has been recorded by attaching a thread to the right ventricle midway between the base and apex of the heart, and leading it to a lever whose movements have been damped by a rubber band. The apex of the heart has been firmly fixed to a rigid bar, the base being fixed by the aortic cannula, so that the lever records the movement of the right ventricular wall, free from swing of the heart. The rate of the heart has been maintained constant by passing rhythmic shocks through fish-hook electrodes, embedded in the right or left ventricle. The importance of maintaining a constant rate of beating, when observations are being made upon the mechanogram, has been clearly shown by Dale [1930]. It is of direct importance in these observations, as certain of the substances have a definite influence upon the rate of beating.

The influence of guanylic acid upon the coronary outflow.

The influence of adenylic acid, both yeast and muscle, adenosine, and guanosine upon the coronary outflow has already been reported [Wedd, 1931; Bennet and Drury, 1931]. The first three substances increase the outflow, while the last has no influence. Guanylic acid, on the other hand, decreases the outflow, though the doses necessary to bring this about are considerably higher than those required for the dilator substances to exert their effect. The result of injecting a series of increasing doses of guanylic acid is shown in Fig. 1, and an injection of 1–2 mg. of the substance invariably leads to a definite decrease in the outflow. Guanylic acid therefore differs essentially in this respect from the other substances already studied, and it can be safely assumed that any change in the mechanogram of the heart consequent upon the introduction of this substance cannot be ascribed to an increase in the coronary flow.

The influence of nucleic acid derivatives upon the mechanogram.

Four substances have been studied, namely, guanylic acid[1], guanosine[1], yeast adenylic acid[1] and adenosine[1]. Muscle adenylic acid[2] has also

[1] Prepared by British Drug Houses, London.

[2] Prepared from ox-heart muscle [Drury and Szent-Györgyi, 1929].

been tested upon one or two occasions. The experiments have consistently shown that in hearts which are beating well after perfusion has been commenced and in which the coronary flow is above 10 c.c. per min., guanylic acid and yeast adenylic acid increase definitely the amplitude of the mechanogram (Fig. 2). The increase in the amplitude is preceded by a decrease, which is of brief duration, but is consistently present. The increased amplitude persists for a considerable time,

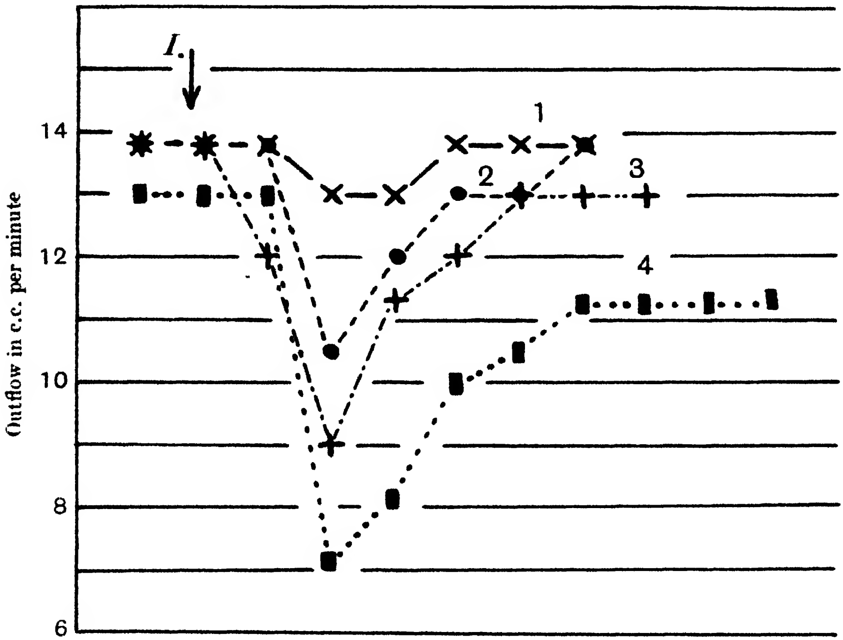

Fig. 1 (1. vii. 16). Influence of guanylic acid upon coronary outflow. Injection of 0·1, 0·5, 1·0 and 2·0 mg. guanylic acid in 1 c.c. of saline at 1, 2, 3 and 4 respectively. Outflow calculated in c.c. per min. from time taken for each successive 5 c.c. to flow through. *I* = injection.

usually 5–10 min., and may persist longer. If further injections are given they lead to a further increase, so that the mechanogram may be 4–5 times as large as it was at the beginning of the experiment, after several injections have been made over the period of half an hour. When guanosine and adenosine are injected no change is seen (Fig. 2). On rare occasions adenosine leads to a slight increase in the mechanogram, which usually occurs when the heart is in poor condition and the coronary outflow is considerably increased by the injection. The absence of change in good preparations is in agreement with the findings

of Wedd [1931]. On a few occasions also a very slight increase has been noted after the injection of guanosine. It is to be noted that the occasional increases seen with both adenosine and guanosine are never preceded by a decreased amplitude. In all these experiments the rate of the heart

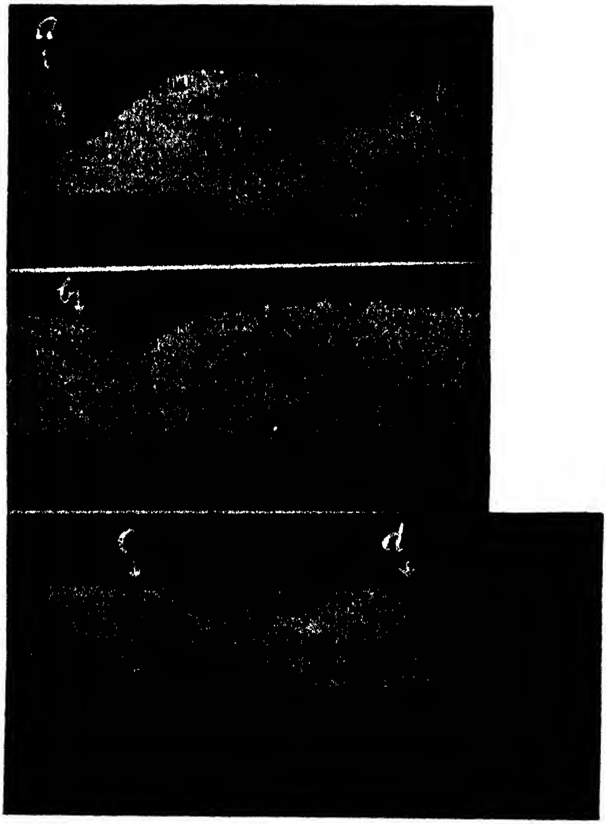

Fig. 2 (1. ix. 13). Influence of nucleic acid derivatives upon amplitude of mechanogram. Injection of 2·0 mg. of yeast adenylic acid, guanylic acid, adenosine and guanosine at *a*, *b*, *c* and *d* respectively. Rate of rhythmic beating = 144 per min. Time marker = 5 sec.

has been maintained constant. If this condition is not fulfilled the rate effect upon the size of the mechanogram is introduced, and this may dominate the result [Dale, 1930]. For instance, adenosine slows the natural rate of beating, and the mechanogram is consequently decreased; guanosine, on the other hand, often enhances the rate of beating, and the mechanogram is thus increased.

In comparing adenylic acid obtained from yeast with the same acid obtained from muscle, no difference has been noted in the general reaction, both producing an initial decrease followed by an increase in amplitude. The results in general suggest that muscle adenylic acid is more effective than yeast adenylic acid, while guanylic acid is the least potent of the three substances.

The substances divide themselves according to these experiments into two very definite groups, those which give rise to a brief decrease, followed by a prolonged increase in amplitude, namely, muscle and yeast adenylic acid, and guanylic acid, and those which are without such influence, namely, adenosine and guanosine. The activity of the first group cannot be due to an influence upon the coronary flow, for while the adenylic acids increase, guanylic acid decreases the flow. In addition adenosine, which is the most efficient dilator, is without influence upon the mechanogram. The changes seen must be associated with some action upon the musculature. In this it is impossible to consider that the ease with which the substances are deaminated comes into play [Drury and Szent-Györgyi, 1929], for adenosine is much more easily deaminated than yeast adenylic acid [Schmidt, 1928]. Chemically the two groups differ from one another in a definite particular. The active substances are composed of a purine base, a sugar and phosphoric acid; the inactive still contain the purine base and the sugar, but the phosphoric acid group has been split off. It would appear that the changes seen in the amplitude of the mechanogram may be due to an effect of this phosphoric group upon the muscle.

To test this point the influence of sodium orthophosphate (di-sodium salt) and pyrophosphate has been examined. It is hardly to be expected that the addition of these substances to the perfusate will produce identical changes with those seen when the phosphoric acid grouping is liberated intracellularly, which occurs when guanylic or adenylic acid is introduced, but it can be hoped that the differences will be in degree only.

Both the substances decrease, in considerable measure, the coronary outflow. Moreover, successive doses appear to have an increasing influence, so that the coronary flow is quickly reduced to such proportions that the preparation fails. This has been obviated in great measure by adding adenosine in concentrations of 1 in 1,000,000 to the perfusate which gives a maximal dilatation of the coronary vessels [Bennet and Drury, 1931] and by using a high perfusion pressure. In these circumstances the first injections produce a decrease in coronary flow which has no detrimental effect upon the preparation. Sooner or

later, however, the coronary flow is greatly reduced, and cannot be increased by heavy doses of either adenosine or nitrites.

Sodium orthophosphate in doses of 0·5–2 mg. leads to an increase in the amplitude (Fig. 3); on rare occasions it has no influence. The curve always lacks, however, the initial decrease which is so characteristic of that produced by adenylic and guanylic acids.

Sodium pyrophosphate, in similar doses, gives rise to an initial brief decrease followed by a prolonged increase in the amplitude. The curve is indistinguishable from that produced by adenylic acid and guanylic

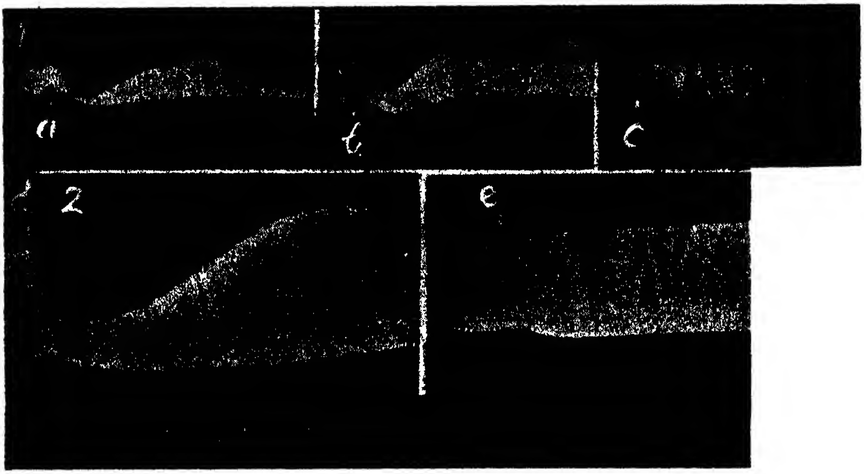

Fig. 3. Influence of sodium orthophosphate (di-sodium salt) and pyrophosphate upon the amplitude of the mechanogram. (1) (1. ix. 26). Rhythmically beating heart. 130 per min. Injection of 2·0 mg. sodium pyrophosphate, 2·0 mg. yeast adenylic acid and 1·0 mg. sodium orthophosphate at *a*, *b* and *c* respectively. (2) (1. ix. 28). Naturally beating heart. Injection of 1·0 mg. sodium pyrophosphate and orthophosphate at *d* and *e* respectively. Time marker 5 sec.

acid (Fig. 3). It would seem, therefore, that the dual effect is associated with a pyrophosphoric acid grouping. The molecular weight of adenylic and guanylic acid is consistent only with the presence of an orthophosphoric acid group, so that it must be assumed that in the intracellular breakdown of these substances either pyrophosphoric acid is formed or that adenylic acid pyrophosphate (and presumably guanylic acid pyrophosphate) is synthesized. This may actually account for the primary brief decrease in the mechanogram. The secondary prolonged increase may be associated with the breakdown of the pyrophosphoric acid or hydrolysis of the adenylic or guanylic acid and the production of

orthophosphoric acid, for this increase is seen when sodium orthophosphate is added.

Parnas and Ostern [1931] have come to the conclusion that adenylic acid and adenine nucleotide pyrophosphate act as "potential poisons" [Loewes, 1928], and that neither deamination with production of ammonia nor other chemical change underlies the reactions. Their observations have been made upon the rhythm of the frog's heart and concern therefore the action of these substances upon the rhythmicity of specialized tissue such as the sinus. The results reported in this paper deal with the influence of derivatives upon the strength of contraction of the muscle of the warm-blooded heart, so that it is not surprising that different conclusions have been arrived at as to their mode of action[1]. The derivatives have many physiological properties; they influence cardiac rhythm, the beat of the heart, the calibre of arteries, intestinal movement, etc. [Drury and Szent-Györgyi, 1929; Bennet and Drury, 1931], and it is unlikely that any one explanation will be sufficient. As further work is undertaken upon the mode of action of these substances the conclusions drawn may appear extremely contradictory unless the varied reactions are clearly appreciated.

It has been noted that adenylic acid is, weight for weight, a less powerful coronary dilator than adenosine [Wedd, 1931], the higher molecular weight of the former being offered as the explanation. The fact that both ortho- and pyrophosphoric acid reduce the coronary flow must be taken into consideration, and the liberation of these substances from adenylic acid, which the described experiments suggest, may decrease the dilator effect of the contained adenosine.

In considering therefore the influence of the nucleic acid derivatives on the beat of the perfused rabbit's heart, two aspects must be considered. Those substances which dilate the coronary system will improve the beat by a more efficient nourishment of the musculature of the heart; those which contain a phosphoric acid group will exercise a beneficial influence through a specific action on the musculature itself.

Influence of various factors upon the action of adenylic and guanylic acid.

Several experiments have been performed in which various changes have been made. On several occasions defibrinated blood from the same animal has been added during the experiment. The amount added has

[1] Whether the conclusions of Parnas and Ostern are applicable to the rhythm changes seen in the warm-blooded heart, it would be out of place to discuss here.

usually been about 10 p.c., and has usually increased the amplitude of the mechanogram. The usual reactions, namely an initial decrease followed by an increase in the mechanogram, have always been observed subsequently upon injecting the two substances. Reducing the calcium content of the Ringer-Locke solution to 0·12 and increasing it to 0·48 p.c. has no effect upon the reaction. After cutting off the supply of oxygen for half an hour, which leads to a definite decrease in the mechanogram, the same general reactions are observed. More complete oxygenation, as provided by the addition of defibrinated blood, has already been noted to leave the reaction unchanged. Atropine added to the perfusate in doses of up to ½ c.c. of a 1 p.c. solution of atropine to the litre is without effect. After the addition of quinine to the perfusate sufficient to reduce the mechanogram from 16 mm. to 5 mm., the same curve is seen when the two substances are introduced. The same result is obtained when the amplitude has been reduced by 50 p.c. by the addition of chloroform. If the substances are added to hearts which have been perfused for 2–3 hours, the usual reaction is obtained and differs little in degree from the reactions obtained in hearts which have been perfused for a short time only.

The whole animal.

In the intact dog, anæsthetized with morphia and chloralose, the mechanogram of the right ventricle has been recorded in the manner already described [Drury, 1923]. Injections of guanylic acid and yeast adenylic acid doses up to 100 mg. have no influence upon the mechanogram. These observations support the conclusions already arrived at for muscle adenylic acid [Drury and Szent-Györgyi, 1929].

Some general reactions of guanylic acid.

Guanylic acid has no influence upon the rhythm of the intact guinea-pig's heart comparable to that produced by adenosine [Drury and Szent-Györgyi, 1929]. Doses up to 5 mg. have no influence upon the rate, but the *T* wave of the electrocardiogram is inverted when high doses are used. The blood-pressure of the intact anæsthetized rabbit is not affected by intravenous injections of 2 mg. of the substance, while doses of 10 mg. give an indefinite rise of pressure.

The virgin guinea-pig's uterus is definitely relaxed when the concentration of the substance in the saline bath reaches 1 in 500,000.

When two drops of a 10 p.c. solution, brought to a *p*H of 7·4 with sodium bicarbonate, are instilled into the rabbit's eye every 10 min. over

a period of 3 hours, no pus can be detected at the inner canthus [Bennet and Drury, 1931].

SUMMARY.

1. In the perfused rabbit's heart, the addition of either guanylic or adenylic acid leads to a brief primary decrease followed by a prolonged increase in the amplitude of the mechanogram; adenosine and guanosine have no such influence.

2. The reaction is not due to an increase in coronary flow as guanylic acid decreases the outflow.

3. Nucleic acid derivatives which contain a phosphoric acid grouping are alone capable of producing the change, and observations with sodium orthophosphate and pyrophosphate suggest that both pyrophosphoric acid and orthophosphoric acid take part in the reaction.

4. In doses up to 100 mg., introduced intravenously, the derivatives have no influence upon the mechanogram of the intact dog's heart.

5. Guanylic acid is without influence upon the rhythm of the intact guinea-pig's heart, and upon the blood-pressure of the intact rabbit. It relaxes the virgin guinea-pig's uterus.

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SOME EFFECTS OF CARBONIC ACID IN HIGH CONCENTRATION ON RESPIRATION.

BY J. BARCROFT AND R. MARGARIA[1].

(*From the Physiological Laboratory, Cambridge.*)

THE first experiments which we shall discuss in the present paper were carried out with the object of ascertaining whether the rate of respiration, which occurred when the animal inhaled air which contained a given percentage of CO_2, was in any way proportional to the initial rate of respiration.

METHOD.

The following experiments were carried out on cats under chloralose. The rate of respiration at the beginning of the experiment was regulated by warming the cat. A tracheal tube was inserted. The cat was then placed in a plethysmograph described by Taylor and figured by Barcroft [1931]. The tracheal tube was joined by a rubber connection to a tube in the door of the plethysmograph. Thus, the animal breathed the outside air, with a dead space little greater than the normal. To this tube could be fitted, when required, a large bag which contained the gas mixture to be administered. The volume of the mixture in the bag was large enough to ensure that no appreciable alteration in composition was caused owing to the fact that a small fraction of it was "rebreathed" by the cat.

RESULTS.

The frequency (*vagi intact*).

The following table shows the effect on the rate of respiration of inhalation of a mixture containing 64 p.c. CO_2, 25 p.c. O_2 and 11 p.c. nitrogen under varying circumstances. Over the range of rates between 20 and 50, the rate during CO_2 administration was clearly not a simple function of the initial rate but was something proper to the fact of CO_2 administration, being almost the same in each of the three cases cited. In the fourth case the respiration during CO_2 was definitely slower than

[1] Rockefeller Fellow.

	Rate of respiration			Total ventilation c.c. per min.			
	Before CO_2	During adminis-tration of CO_2	Per-centage alteration	Before CO_2	During CO_2	Per-centage increase	Remarks
1	50	27	−46	415	863	108	Cat warmed
2	26	29	+12	572	1292	126	Cat again warmed
3	21	26	+24	588	1289	119	Cat again warmed
4	12·3	19	+34	407	948	133	Cat not warmed

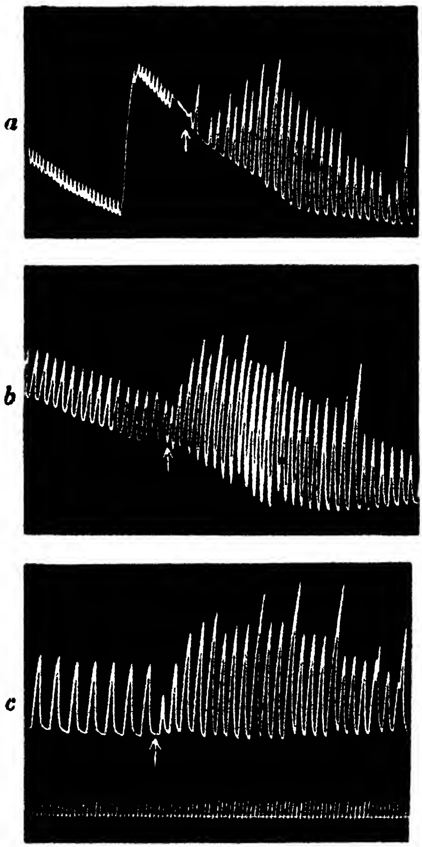

Fig. 1. Showing the effect of inhalation of CO_2 on the rate of respiratory rhythm. Arrow indicates moment of CO_2 administration. *a*, rapid initial rhythm. The falling base line is due to cooling of the air in the plethysmograph. The apparent interruption of the rhythm immediately preceding the arrow is an artefact due to pinching the inhalation tube. *b*, initial rhythm slower than *a*. *c*, very slow initial rhythm. Time in seconds.

the others. This may have been due to the cat being at a lower temperature. In any case it is clear that the effect of CO_2 in high concentrations may be either to quicken or slow the breathing markedly according to the rate and nature of the initial rhythm. Whilst there is not sufficient uniformity in the rhythm to justify quantitative treatment, the general result indicated by six tests that there is a frequency in the region of 30–35 which is proper to CO_2.

In greater detail the effect of inhalation of so strong a concentration may be described as follows:

Usually when CO_2 is administered a certain picture appears, the rate suddenly changes from its original value, takes on a value a little below 30, rises to somewhere about 35 and then falls off again. If, therefore, the initial rate is about 35, it is likely to be very little altered by 60 p.c. CO_2 and the duration of the alteration cannot be predicted precisely.

The interesting points are the relative uniformity of the CO_2 type of response and the fact that the original rhythm, imposed in some of our experiments presumably by the thermogenic central mechanism, is wiped out.

The depth of respiration and the total ventilation (vagi intact).

The effect of 60–65 p.c. CO_2 on the depth of respiration and on the total ventilation is always to increase both for a short time. The depth of respiration reaches a fairly constant maximal value, which in our experiments has been about 60–70 c.c. Probably the animals could achieve little more.

The increase in amplitude is, however, transitory: with the high concentrations of CO_2 which we have used, it begins to fall off in about half a minute, the frequency being maintained. The alterations in total ventilation, therefore, tend to reflect those in amplitude. If the administration of 60–65 p.c. CO_2 had been persisted in, the result would shortly have been fatal. In most cases we allowed the cat to revert to air before that result ensued, but after the first half minute the respiration declines and with the vagi uncut it is not very easy to say what takes place. The general impression gained from watching the process is that (1) the frequency decreases slightly, (2) the normal ordered respirations decline in height, but a gasp is often imposed upon the previous rhythm. Therefore a rhythm appears similar to that shown in Fig. 2*b*.

As this process continues the respirations disappear entirely and only the gasps remain. Ultimately, the gasp weakens and death supervenes unless the CO_2 be removed.

Effects with the vagi cut.

If the vagi have been cut, there is usually little change in the rhythm on giving CO_2 as was found by Scott [1908]; otherwise most of the phenomena which we have described are seen more clearly than with the vagi intact. Some other phenomena of interest appear. Between the stage of hyperpnœa and that of gasping there is always a tendency to apneusis. In some animals it is trifling and it depends upon the anæsthetic—with chloralose we have never failed to get indications of it.

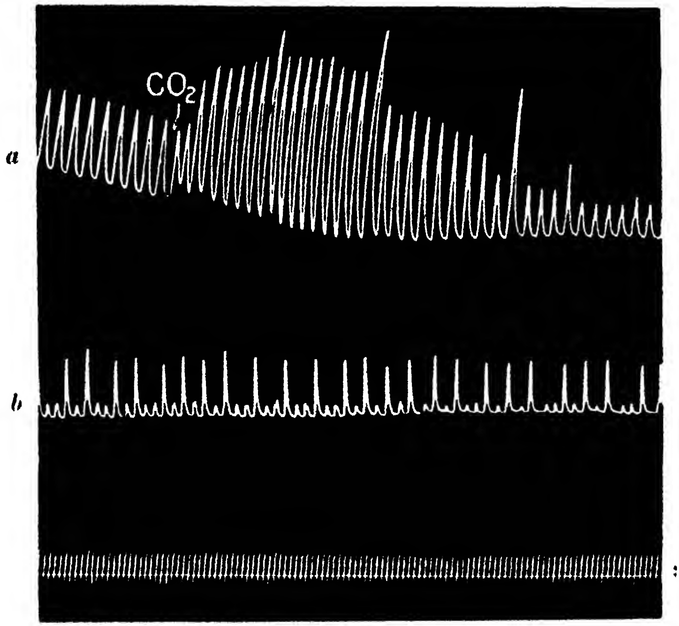

Fig. 2. *a* and *b* are parts of a continuous tracing showing the earlier and later stages in administration of 60 p.c. CO_2. There is an interval of 4 min. and 5 sec. between the end of *a* and the commencement of *b*. The distinction between the respiration and the imposed gasp is much more evident to one observing the experiments than it is on the tracing. The lever rises rather slowly at first in such a respiration as the fifth in *b*, and then very rapidly.

Chloroform is the anæsthetic least favourable, in our experience, to a positive result. On the other hand, the apneuses are very pronounced in cats anæsthetized with dial. Thus the whole train of stages obtained by Lumsden [1923] as the result of sections of the medulla from above downwards can be obtained by the administration of strong CO_2, and

this even though the percentage of oxygen is much above that normally in the atmosphere.

H. Taylor [1930] has shown that this series of respiratory effects is also produced by the inspiration of air which contains hydrocyanic gas in small quantities. It became of great interest to us to see whether, on the same animal, we could get the hyperpnœa, the apneuses, and the gasps first by the administration of CO_2 and subsequently by the administration of HCN. The experiments for the test took place in a

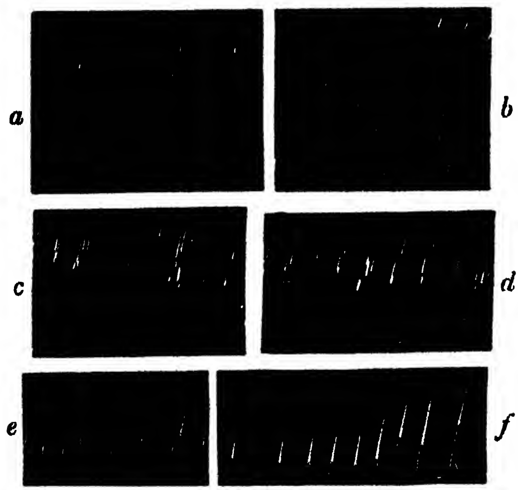

Fig. 3. *a–f*, successive alterations in respiration induced by CO_2—*a*, normal; *b*, hyperpnœa; *c* and *d*, apneuses; *e* and *f*, gasps. Chloralose anæsthesia—vagi cut.

glass respiration chamber of approximately 10 cubic metres capacity. The CO_2 mixture was given from a bag as already described. The mixture was given twice, the interval being approximately 5 min. from the reversion to air after the first test to the commencement of CO_2 respiration in the second. The tracing shown in Fig. 4 is the second of the two; in all essentials the first was similar, so that the effects were reproducible on the animal. When the respiration had recovered, liquid HCN was placed in the chamber in front of a fan, so that it evaporated immediately and was distributed as nearly uniformly as possible, the result is shown in Fig. 4 *c–d*. The essential alterations in the type of respiration are the same for both gases, initial increase in amplitude, apneuses, gasps, though in the case of the HCN, with the dose given the gasps became separated by longer intervals of time and indeed the result was fatal.

Summary.

1. Inhalation of atmospheres rich in carbonic acid tends to produce a certain characteristic type of respiration which may be either faster or slower than the rhythm which existed previously depending upon the rate of the initial rhythm.

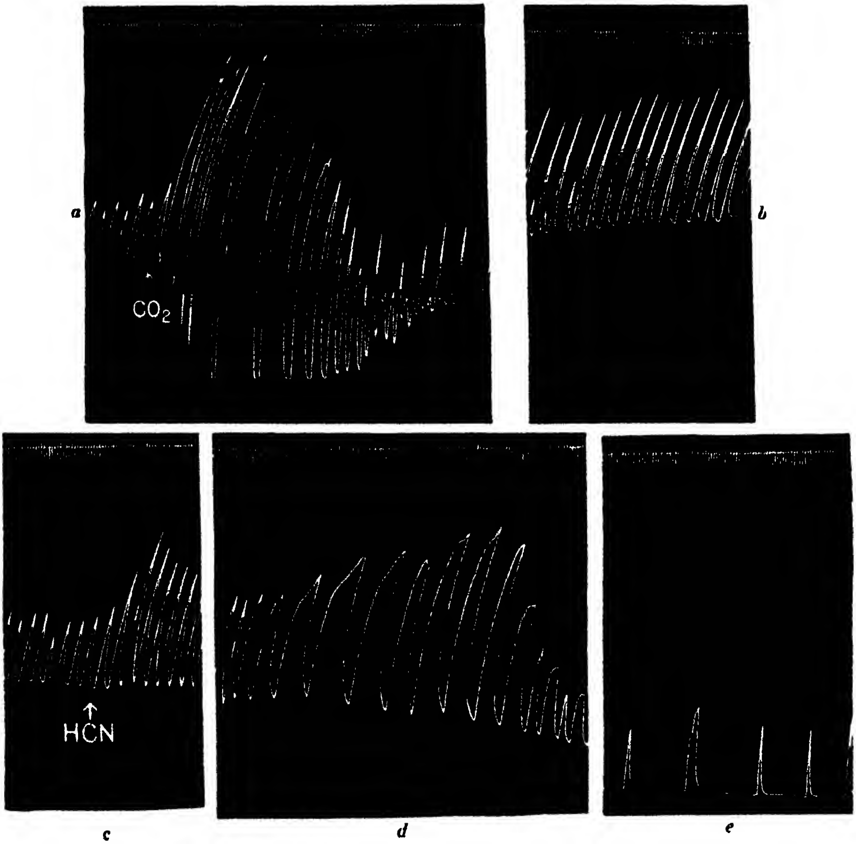

Fig. 4. Cat, dial, *a* and *b* effects of administration of 60 p.c. CO_2; the end of *b* corresponds with the cessation of CO_2; cat breathed air between *b* and *c*. *c*–*e*, effects of administration of HCN about 17 mg. per litre of air. Intervals: between *a* and *b* 2 min. 21 sec.; between *c* and *d* 2 min. 10 sec., in which there were some irregularities; between *d* and *e* 1 min. 10 sec.

2. In cats with the vagi cut, and especially under dial hypnosis, high concentrations of CO_2 will produce the train of symptoms observed by Lumsden and resulting from successive sections of the brain, and

also by Taylor as the result of HCN administration: pneumotaxis, apneusis, gasping, standstill.

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THE METABOLISM OF POSTURAL AND PHASIC CONTRACTIONS OF THE QUADRICEPS OF THE CAT.

BY E. L. WEATHERHEAD.

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I. INTRODUCTORY.

THE metabolism of mammalian muscle in postural contraction has been studied in decerebrate preparations by Dusser de Barenne and Roaf. Their results are rather contradictory, since Roaf [1912, 1913] finds no significant increase over the resting value, and Dusser de Barenne and Burger [1924] find a distinct increase.

Recently it has been found possible to keep a muscle in postural contraction for several hours [Briscoe, 1931]. At the suggestion of Prof. Roaf it was decided to study the metabolism of an animal when maintaining such a contraction, comparing it with its metabolism in rest, and when making regular "phasic" movements such as occur in walking.

In the work on decerebrate animals a control (resting) metabolism was possible only at the end of the experiment. With this present method, however, the resting metabolism can be estimated before as well as after any posture or movement, and thus an additional control be obtained.

II. EXPERIMENTAL METHODS.

(a) *Methods of producing the contraction.*

A method of obtaining in a muscle full postural shortening which will endure without sign of fatigue for prolonged periods has already been described [Briscoe, 1931].

Summarized, the method consists in submitting the nerve of the selected muscle or group of muscles to a relatively low rate of stimulation (18–25 per sec.) under certain experimental conditions (notably full blood supply and light anæsthesia). The iterative stimuli were supplied by the

regular discharges from a neon lamp flashing circuit [Briscoe and Leyshon, 1929].

With a rate of stimulation within these limits and using this method a muscle can be maintained in postural contraction for as long as 3 hours, and probably could be so held for a much longer period.

Stimulation at more rapid rates maintaining a constant strength produces the familiar quickly tiring "tetanus," but if the strength of these faster rates be rhythmically increased and decreased (within the limits of threshold and submaximal values) then rhythmic ("phasic") movements such as normally occur in the acts of walking or running will be produced.

(*b*) *Method of estimating the metabolism.*

The animal was anæsthetized by "dial" (diallyl barbituric acid and urethane) using 0·5 c.c. per kg. of body weight. It was placed on a warm well-padded table.

The rectal temperature of the animal was noted and every effort made to keep this as constant as possible.

A tracheal cannula was inserted, and on to this were fixed inlet and outlet valves made of rabbit gut similar to those described by Pearce (in Macleod's *Physiology*, 1927). These valves offered no resistance to breathing and proved very efficient. The outlet valve was connected with a rubber T-piece, one limb of which led immediately to a sampling tube, and the other limb to a 6-litre bell spirometer. Thus the expired air could be sampled and measured over a period of 5 to 10 min. (Fig. 1).

The sciatic and femoral trunks in both limbs were exposed and cut. Branches of the femoral supplying other muscles than the quadriceps were also cut. All the operative work in these experiments was carried out by G. Briscoe. After the completion of operative procedures the animal was allowed to rest completely for half an hour. The resting metabolism was then estimated in the following manner:

The sampling tube and connecting tube (*A*) were filled with mercury and the spirometer emptied of air. By turning tap *B* the animal was connected with the spirometer.

The tap was of a large calibre and offered no resistance. A stop-watch was simultaneously started. By partially opening the tap of the sample tube the mercury could be withdrawn very slowly and a sample of expired air be drawn into the tube. Samples could thus be taken over as long a period as was desired. When the sampling tube was full, the taps were turned off, the time taken and the spirometer level noted.

After one or two resting samples had been taken, the legs were put into either phasic or postural contraction and further samples collected, allowing always at least 5 min. between the beginning of a contraction

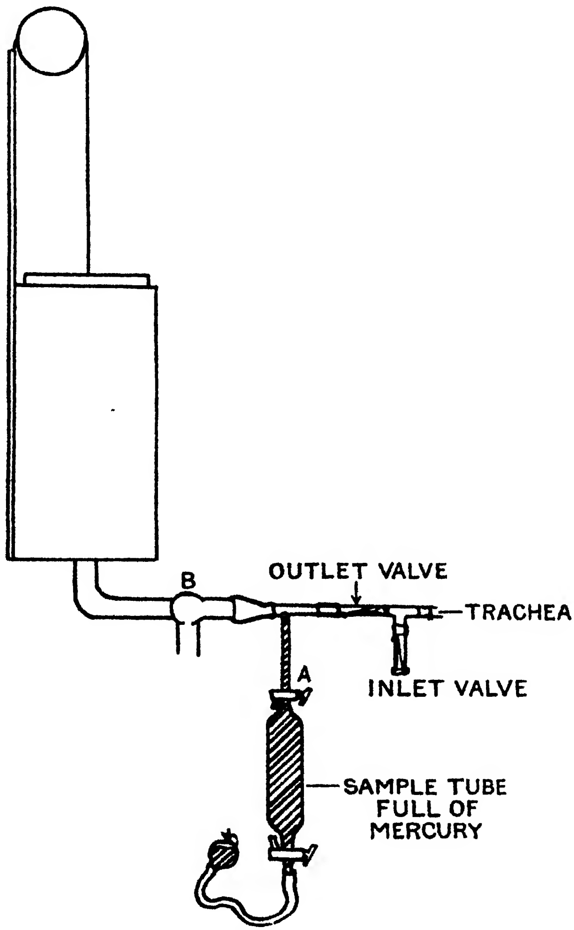

Fig. 1. Diagram of apparatus.

and the taking of a sample. After phasic contraction the animal was always given a rest of at least 20 min. before any other estimation was made. In most experiments a resting sample was taken at the end of this period in order to test whether the metabolism had returned to the resting level.

Table I. Respiratory exchange (c.c. per minute at N.T.P.) in rest, phasic movement and posture.

Date	(1) Resting			(2) Resting			(3) Phasic			(4) Resting			Time of start of post.	(5) Posture			(6) Posture			(7) Posture			(8) Resting			Range of temp. var. °C.
	Time	O_2	CO_2	Time	O_2	CO_2	Time	O_2	CO_2	Time	O_2	CO_2		Time	O_2	CO_2	Time	O_2	CO_2	Time	O_2	CO_2	Time	O_2	CO_2	
27. x 30	12.30	11·6	7·5	12.45	11·1	7·3	2.47*	15·4	12·6	—	—	—	1.0	1.10	14·8	10·8	1.47	11·3	8·2	—	—	—	2.20	10·3	7·4	0·2
17. xi. 30	1.40	14·9	9·9	1.50	15·7	10·8	4.20*	15·3	11·2	—	—	—	2.15	2.45	15·4	10·6	3.35	14·6	10·4	—	—	—	3.55	13·9	9·9	1·0
26 i. 31	12.45	19·0	16·1	12.54	19·6	15·7	1.55*	25·0	19·3	—	—	—	1.10	1.20	25·6	21·5	—	—	—	—	—	—	—	—	—	0
9. ii. 31	2.13	20·8	15·0	—	—	—	6.12*	22·0	16·2	—	—	—	2.20	2.35	26·5	18·8	3.15	25·4	18·4	4.15, 5.15	24·4, 25·5	18·2, 19·1	5.50	19·8	15·1	0·6
23. ii. 31	2.3	29·9	21·2	2.12	30·2	21·5	2.30	39·9	29·3	2.55	27·2	19·6	3.3	3.13	35·5	26·2	4.13	32·7	24·7	5.0	32·9	24·3	5.14, 5.30	25·9, 28·2	19·1, 20·1	0·5
4. v. 31	1.30	20·4	15·1	1.43	20·7	15·3	2.0	27·8	22·6	2.55	19·1	14·0	2.35	2.47	24·4	18·6	3.30	22·5	16·6	—	—	—	5.45	19·4	14·4	0·5
11. v. 31	11.25	15·3	11·9	11.37	14·8	10·9	11.58	19·9	16·5	1.25	13·8	10·6	1.40	1.49	14·7	11·4	2.40	14·2	10·8	3.36	14·4	10·7	3.55	13·1	10·1	0·7
1. vi. 31	12.10	18·3	12·4	12.25	17·8	12·2	12.50	28·5	20·7	1.55	16·8	12·2	2.10	—	—	—	3.40	23·9	16·8	3.50	24·0	17·5	4.5, 4.20	18·7, 18·0	13·2, 12·1	0·9
Control experiment—resting all the time:																										
15 vi. 31	12.35	14·4	9·7	12.50	14·0	9·7	1.35	13·5	8·7	1.50	13·3	8·8	—	2.35	13·5	9·8	3.35	13·2	9·9	3.50	13·1	9·1	—	—	—	0·3

* In these experiments phasic movement was done at the end of the experiment. The resting metabolism for these figures is therefore found in column 8.

(c) *Reliability of the method.*

The first two columns of Table I give a series of duplicate readings, and it will be seen from them that the variation between two consecutive estimations is below 10 p.c., and in all but two cases below 5 p.c. or less.

Changes greater than 10 p.c. may therefore be considered significant—possibly even changes less than that.

The greatest difficulty in technique was that of keeping the animal at a constant temperature. In these experiments the variation of temperature was never more than 1° C., and in several considerably less.

(d) *"Normality" of the experiments.*

No experiments on anæsthetized animals can be considered quite normal. The questions to be considered are (i) whether the animal is a dying animal, and (ii) whether the anæsthetic itself is tending to produce an abnormal metabolism.

(i) The "benignity" of dial anæsthesia is well known. Bickel and Katzenelbogen [1924] record cases in which patients attempting suicide by taking 10 to 25 times the usual therapeutic dose only fell into a deep sleep lasting several days.

In these experiments the degree of anæsthesia was not deep enough to abolish the corneal reflex, and it seems probable that the animals would have been capable of recovery, had they not been killed at the end of the experiment.

On the other hand there is a slight but probably definite fall in the resting metabolism throughout the experimental period. The extent of this fall is shown in Table I and in Fig. 2. The experiment of June 15 shows the resting metabolism over an undisturbed 3½ hours' period. The other experiments show a slightly lower resting metabolism at the end of the experimental period. Though most of these values fall within the 10 p.c. range of variation, not all of them do so.

On these data then, we think it justifiable to assume a small fall in metabolism over a 3–4 hour experiment.

(ii) The effect of diallyl barbituric acid on the metabolic processes is not known.

Work on "amytal" (isoamylethyl barbituric acid) has shown that although this drug apparently does not affect the blood-sugar concentration [Mulinos, 1928] it does slowly deplete the liver of its glycogen [Evans, Tsai and Young, 1931]. The pharmacological resemblance

of "dial" and "amytal" makes it probable that animals under "dial" anæsthesia would have a similar abnormality of metabolism.

Some slight evidence on this point may be obtained from the respiratory quotients in these experiments. Table II gives the R.Q.'s in the

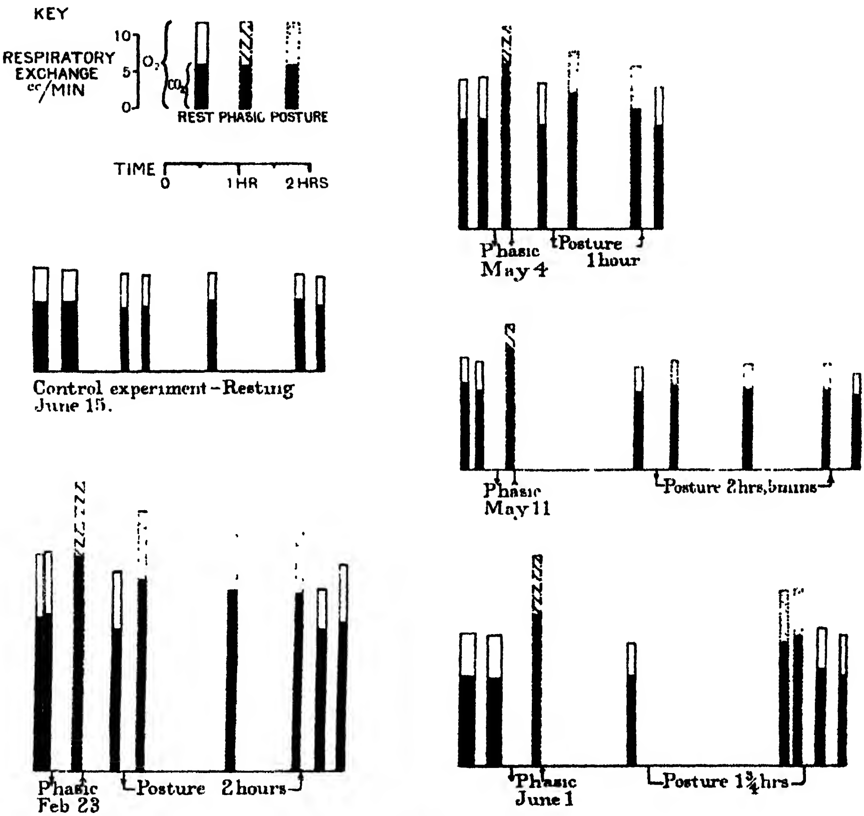

Fig. 2.

TABLE II. Respiratory quotients.

Date	Resting: At start of exp.	Resting: At or towards end of exp.	During phasic contraction	During postural contraction
27. x. 30	0·65, 0·66	0·72	0·81	0·73
17. xi. 30	0·67, 0·69	0·71	0·73	0·69
26. i. 31	0·84, 0·80	—	0·77	0·84
9. ii. 31	0·72, 0·71	0·76	0·74	0·71, 0·73, 0·75
23. ii. 31	0·71, 0·71	0·73, 0·72	0·74	0·74, 0·75, 0·74
4. v. 31	0·74, 0·74	0·74	0·81	0·76, 0·74
11. v. 31	0·78, 0·74	0·77	0·83	0·78, 0·76
1. vi. 31	0·67, 0·69	0·71, 0·69	0·73	0·71

resting condition and during postural and phasic contraction (irrespective of the time in the experimental period at which these were determined).

The R.Q.'s during the control resting experiment were as follows: at the beginning of the experiment 0·68, 0·69; after 1 hour 0·64, 0·66; after 2 hours 0·73; after 3 hours 0·75, 0·69.

It will be seen that in four out of nine experiments the R.Q. is below 0·7 at the beginning of the period, though in all it is slightly higher at the end. In all but one experiment the R.Q. rises definitely with phasic contraction, and to a smaller extent with the postural contraction.

III. Results.

In all, fifteen experiments on metabolism have been carried out. In the first series of six experiments when only one quadriceps was used, the rises in metabolism during postural contraction were too small to be regarded as definitely outside the limits of experimental error. In the second series both quadriceps muscles were used; all figures and tables refer to this second series. The figures are given in Table I. The results for four experiments and the control are shown graphically in Fig. 2.

(a) *Metabolism during postural contraction.*

Estimations of the metabolism were made at different stages in the postural contraction. These will therefore be considered under (i) metabolism at the beginning of posture, and (ii) metabolism during a prolonged posture.

(i) Column 5 of Table I gives the metabolism 10–15 min. after the beginning of posture. In order to compare these figures more easily, the O_2 consumption and CO_2 output are expressed below in Table III as percentages of the resting values immediately preceding them. (In the experiment of Nov. 17 the first estimation was not made till half an hour after the beginning of posture.)

Table III.

Date	O_2 consumption p.c.	CO_2 output p.c.
27. x. 30	128	144
26. i. 31	134	134
9. ii. 31	127	125
23. ii. 31	130	133
4. v. 31	128	133
11. v. 31	106	108

In five out of these six experiments the O_2 consumption has risen to approximately 130 p.c. of its resting value immediately before the

stimulation. Similar but more irregular results are obtained from the CO_2 figures.

(ii) Metabolism during a prolonged posture is shown in columns 6 and 7 of Table I. These O_2 consumptions are grouped together and compared as percentage of previous resting value in Table IV.

TABLE IV. O_2 consumption: percentage of preceding resting value.

Date	Time after beginning of posture				
	10 min.	30–45 min.	1–1½ hours	2–2½ hours	3 hours
27. x. 30	128	98	—	—	—
17. xi. 30	—	104	98	—	—
9. ii. 31	127	—	122	117	122
23. ii. 31	131	—	120	121	—
4. v. 31	128	—	118	—	—
11. v. 31	106	—	103	104	—
1. vi. 31	—	—	142	143	—

Figures from four of these experiments are shown diagrammatically in Fig. 2. These figures are not nearly so constant as those at the beginning of posture. Three of the seven experiments show a metabolism of approximately 120 p.c. of the resting, 1 hour after the beginning of posture. Three experiments show a fall to approximately the resting value and one experiment shows a rise to 140 p.c. (In this experiment an unusually high rate of 28 a sec. was used for part of the time.) In the experiments in which posture has been held for 2, 2½ and 3 hours, no further fall is shown.

On the whole then, the experiments show a definite increase of metabolism at the beginning of posture.

It is probable that this is maintained with only a slight fall (which may itself be due to a decrease in metabolic activity of the animal as a whole).

(b) *Metabolism after postural contraction.*

It is characteristic of postural contractions obtained under these conditions to show no sign of fatigue. When the stimulus is stopped the muscle relaxes very slowly, doing so in a series of "steps." This relaxation can be hurried by giving the limb a few "passive movements," when it will at once assume the relaxed position.

If there were any accumulation of lactic acid during posture it should be shown in the resting metabolism immediately after. A comparison of columns 8 and 1 in Table I shows, however, that the metabolism returns within 10 min. to, or below, the original resting value. This is also shown in Fig. 2.

(c) *Metabolism of phasic contraction.*

As far as one knows, there are no published experiments comparing the postural and phasic activity of the same muscle and that muscle only. Dusser de Barenne and Burger [1924] state that "although this augmentation of the respiratory exchange under decerebrate rigidity is distinct, it is much less than the augmentation of the respiratory exchange during phasic innervations and movements."

Roaf [1913] states that "abolishing the rigidity of the muscles in the decerebrate cat reduces the oxygen intake to 98·8 p.c. of what it was whilst the muscles were rigid," and finds that the average respiratory ratio was 18 p.c. greater in those experiments in which slight, discontinuous muscular movements occurred.

In our experiments the movements obtained were rhythmic and regular, in rate from 2 to 3 per sec. These could be obtained on very variable "basic rates" of stimulation varying from 50 to 275 stimuli per sec. (No relation was found between the "basic rate" and the metabolism.)

From Table I, column 3, and Fig. 2 it will be seen that in two experiments (October 27 and June 1) the O_2 consumption is 150 and 156 p.c. of resting. In four experiments it is 131, 133, 136 and 131 p.c. of resting, while in the remaining two experiments it is only 110 and 106 p.c.

The greatest increase in CO_2 output is (on October 27) 205 p.c. and the lowest (February 9) 108 p.c.

These results are surprisingly low. In considering the relation of postural and phasic contraction we are accustomed to fall back on the comparison of such activities as standing and walking—*e.g.* the figures given by Douglas and Haldane [1912] where (expressing the figures as percentages) the O_2 consumption standing is 138 p.c. of that at rest in bed, and during a period of walking at 2 m.p.h. is 282 p.c. of the resting.

But in such an activity as walking, more than the postural muscles are employed, and a truer view we think is obtained of the intrinsic relation of postural to phasic activity in the present experiments where the metabolism of the *same muscles* in these different activities can be compared. We are therefore forced to the conclusion that regular phasic movements can be carried out with a comparatively low metabolic cost, sometimes (with small movement) indistinguishable from the cost of postural contraction.

SUMMARY.

1. A method is described for estimating the metabolism of postural and phasic contraction of the quadriceps of the cat.

2. A definite increase of metabolism usually accompanies postural activity. In most experiments this, expressed in terms of O_2 consumption, was about 130 p.c. of the resting, though in some experiments it was evident that posture could be maintained at a much lower cost hardly to be distinguished from the resting metabolism.

3. The metabolism during a prolonged postural contraction falls slightly, though it is possible that this is due to the general slight fall in metabolism over a 3–4 hour experiment.

4. Directly the postural stimulus is stopped the metabolism returns to the resting value.

5. There is a definite but variable increase in metabolism during phasic contraction, the greatest O_2 consumption being 156 p.c. of resting, the least 106 p.c.

I wish to express my thanks to Prof. Cullis for her interest and advice, and to Lady Briscoe for much valuable help and criticism throughout the work.

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612.621:612.018

STUDIES ON OVULATION.

VI. Relative importance of concentration and absolute amount of the ovulation-producing hormone.

By F. W. ROGERS BRAMBELL and A. S. PARKES
(*Foulerton Student of the Royal Society*).

(*From the Department of Zoology, University College, Bangor.*)

I. Introduction.

It is now reasonably certain that ovulation follows copulation in the rabbit as the result of reflex stimulation of the hypophysis causing secretion of the ovulation-producing hormone by the anterior lobe. This combination of nervous and endocrine events suggested that instructive results might be obtained from cross-circulation experiments, *i.e.* from the anastomosis of a normal copulated animal with a hypophysectomized unmated œstrous one[1]. Before such experiments could be carried out, however, it was necessary to find an anti-coagulant with no effect on ovulation, and to investigate the effect of lowering the concentration of the ovulation-producing hormone by dispersing it into a double circulation. The first problem has been dealt with in the preceding paper [Brambell and Parkes, 1932]: the second is considered in this one.

It was evident from earlier work [Fee and Parkes, 1929] that the whole amount of the hormone required to produce ovulation is secreted by the anterior pituitary body within 1 hour after copulation; it was also a reasonable assumption that the major part of the hormone is in circulation during the following 30 min. It seemed, therefore, that the concentration of the hormone could be altered by replacing a proportion of the blood with gum saline. Such a technique, however, would also lower the absolute amount of the hormone and introduce a second variable. To eliminate this complication, *i.e.* to lower the concentration without altering the absolute amount per follicle, it was necessary to remove a corresponding proportion of the follicles. If a greater proportion of follicles than of blood was removed, the absolute amount of hormone

[1] This idea was originally elaborated by the late Dr A. R. Fee, who actually carried out one experiment before his death.

available per follicle could be raised above normal, and *vice versa*. A long series of experiments was carried out on these lines and, as might be expected from their nature, the details of the results are not very coherent. In their main outline, however, the experiments are quite decisive, and give the somewhat surprising result that the concentration of the hormone can be lowered very considerably without necessarily inhibiting ovulation.

Our experiments on hormone concentration and on the use of Chicago blue as an anti-coagulant, suggest that it will be possible to carry out cross-circulations for the required time. A variety of purely technical difficulties have arisen, especially diffuse hæmorrhage from cut surfaces, but preparations have already been maintained for more than 9 hours.

The present paper is concerned solely with the effects of altering the concentration of the ovulation-producing hormone in single rabbits.

II. Methods and material.

Œstrous rabbits were obtained as previously described and a preliminary laparotomy performed to ascertain the number of ripe follicles. Anæsthesia was induced as before.

Calculation of the total blood volume. Before a definite proportion of the blood could be removed it was necessary to calculate the total blood volume of the animal. So far as could be ascertained the most complete data on blood volumes are those of Dreyer and Ray [1910] who give a curve for the blood volume of rabbits according to weight. This curve was used throughout our experiments.

Calculation of the amount of blood removed. It was clearly undesirable that an appreciable percentage of the total blood of an animal should be removed at once. It was decided, therefore, to obtain the required amount in steps; fluid equal to 5 p.c. of the original blood volume being removed at a time and a similar quantity of gum saline replaced immediately. In an animal with a blood volume of 100 c.c., therefore, 5 c.c. would be removed each time and 5 c.c. gum saline replaced. The first 5 c.c. removed would be undiluted blood, but the second 5 c.c. would be $5 \times \frac{19}{20}$ c.c. blood plus $5 \times \frac{1}{20}$ c.c. gum saline. Thus, after the second removal, 90·25 c.c. original blood would remain in the animal. The third 5 c.c. would only contain $5 \times \frac{19}{20} \times \frac{19}{20}$ c.c. blood, leaving 85·74 c.c. still in the animal. The amount of blood remaining after each removal and replacement by gum saline is shown in Fig. 1.

To remove 50 p.c. of the blood it was therefore necessary to remove

5 p.c. of the circulating fluid and replace with gum saline thirteen and a half times; to remove 40 p.c. to make ten removals, etc.

Bleeding technique. The ripe follicles were counted and one ovary or the other extirpated, according to the proportion of follicles it was desired to remove. Bleeding was then carried out from a carotid, gum saline being run into the opposite jugular immediately after each removal of blood. No cannula was put into the carotid: an inch or more

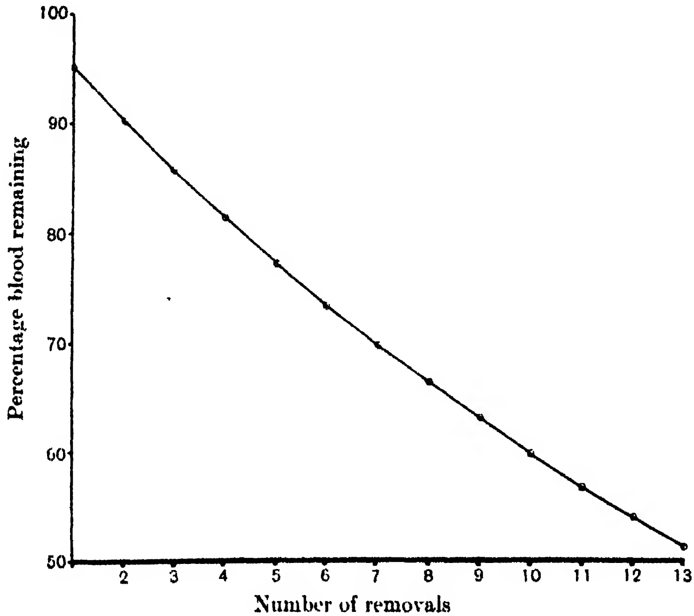

Fig. 1. Amount of original blood remaining after successive removals of 5 p.c. of the circulating fluid and replacement with gum saline.

of the artery was dissected out and severed, so that the stream of blood could easily be directed into the measuring cylinder.

Control experiment. As a control on the possibility of hæmorrhage affecting the occurrence of ovulation, an animal was given an injection of the ovulation-producing substance (3 c.c. urine of pregnancy) immediately after the replacement of 50 p.c. of the blood by gum saline, both ovaries being left in. Ovulation took place at about the normal time, showing that this amount of bleeding is not incompatible with ovulation provided that an adequate amount of hormone is available.

III. Experimental results.

It is evident that the technique described above is subject to a number of variable factors. Chief among these would appear to be individual variation in (*a*) the amount of surplus hormone, (*b*) the general reaction to the bleeding process, (*c*) the exact time after copulation at which the ovulation-producing hormone reaches its maximum concentration in the blood, and (*d*) the proportion of ripe follicles which would have ovulated without treatment. Also, difficulty was experienced in some animals in determining the number of mature follicles. In these circumstances no great coherence could be expected in the results, but it is possible to arrive at certain conclusions from the thirty satisfactory

Table I. Effect of altering the concentration and the absolute amount available per follicle of the ovulation-producing hormone after copulation in the rabbit.

No. of animal	Ripe follicles: Total number	Ripe follicles: Number left in	Absolute amount of hormone per follicle	Percentage blood left in, *i.e.* concentration of hormone p.c.	Result
CCB 20	9	9	0·9	90	9 follicles ovulated
CCB 16	8	7	1·0	90	7 ,,
CCB 15	5	3	1·5	90	No ovulation
CCB 52	7	7	0·8	80	No ovulation
CCB 11	10	5	1·6	80	1 follicle ovulated
CCB 22	11	11	0·7	70	11 follicles ovulated
CCB 48	10	7	1·0	70	No ovulation
CCB 19	5	3	1·2	70	,,
CCB 42	7	4	1·2	70	,,
CCB 50	8	4	1·4	70	1 follicle ovulated
CCB 9	7	3	1·6	70	1 follicle ovulated
CCB 52	5	2	1·7	70	No ovulation
CCB 47	8	8	0·6	60	No ovulation
CCB 39	5	3	1·0	60	,,
CCB 8	5	3	1·0	60	,,
CCB 18	4	2	1·2	60	2 follicles ovulated
CCB 37	8	4	1·2	60	No ovulation
CCB 51	7	3	1·4	60	,,
CCB 49	5	2	1·5	60	,,
CCB 45	8	3	1·6	60	1 follicle ovulated
CCB 6	2	2	0·5	50	No ovulation
CCB 17	6	5	0·6	50	,,
CCB 29	8	5	0·8	50	,,
CCB 31	7	4	0·9	50	,,
CCB 26	6	3	1·0	50	,,
CCB 7	13	5	1·3	50	,,
CCB 28	9	3	1·5	50	1 follicle ovulated
CCB 24	7	2	1·7	50	2 follicles ovulated
CCB 35	8	2	2·0	50	No ovulation
CCB 30	9	2	2·2	50	1 follicle ovulated

experiments performed. The details are recorded in Table I and a summary in Table II. The following conclusions may be drawn:

(*a*) Reduction of the concentration of the hormone down to 70 p.c. does not necessarily inhibit ovulation, even when the absolute amount of hormone per follicle is also reduced. CCB 22, for instance, ovulated eleven follicles with both the concentration and the absolute amount of the hormone lowered to 0·7 normal.

(*b*) Reduction of the concentration to 60 p.c. of the normal or less does, however, seem to inhibit ovulation under the conditions obtaining, unless the absolute amount of hormone available per follicle is raised by the removal of a large proportion of the original mature follicles.

The second of these conclusions might be explained on the grounds that: (*a*) the rate at which a follicle could withdraw the hormone from the blood would be proportional to its concentration; (*b*) the more follicles present, the more rapidly would the initial concentration sink to a level at which withdrawal would be too slow to permit of ovulation in the required time. Therefore, with a low initial concentration, the absolute amount available per follicle must be greater than normal so that the concentration does not sink prematurely below the effective point.

TABLE II.

Absolute amount per follicle	Concentration of hormone: 70 p.c. normal or more — No. of experiments	No. ovulating	60 p.c. normal or less — No. of experiments	No. ovulating
Less than normal	3	2	5	0
Normal	2	1	3	0
More than normal	7	3	10	5
Total	12	6	18	5

As regards cross-circulation experiments, the non-copulated rabbit must be supposed to have a certain amount of the hormone in the blood, and therefore anastomosis will not result in a concentration as low as one-half that originally present in the copulated animal. Nevertheless, it is probable that care will have to be taken to reduce the number of follicles present.

IV. SUMMARY.

1. The concentration of the ovulation-producing hormone in the circulation of the rabbit after copulation was altered by the removal of varying amounts of blood and replacement with gum saline. The absolute amount of hormone available per follicle was adjusted by the removal of a varying proportion of the ripe follicles.

2. Reduction of the concentration by up to 30 p.c. does not necessarily

inhibit ovulation, even when the absolute amount per follicle is also allowed to decrease coincidently.

3. Reduction of the concentration by 40 p.c. or more inhibits ovulation under the conditions obtaining, unless the absolute amount per follicle is raised by the removal of a large proportion of the original ripe follicles.

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612.328:612.893]615.781

THE SYMPATHETIC INNERVATION OF THE STOMACH.

IV. Reversal of sympathetic action by luminal.

By G. L. BROWN and B. A. McSWINEY.

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In 1930, Brown, McSwiney and Wadge showed that either contraction or relaxation of the stomach could follow stimulation of the thoracic sympathetic trunk in the spinal cat. Relaxation was the usual response, but contraction of the stomach could be produced by stimulating with very low frequency or with very weak stimuli. Later, McSwiney and Robson [1931 *a*] were able to obtain identical results from stimulation of the peri-arterial nerves supplying isolated strips from the fundus of the stomach of the cat and rabbit. They brought forward evidence to show that the nerves stimulated were postganglionic sympathetic, and that their results were not due to the stimulation of admixed fibres of vagal origin.

In our preliminary experiments it was found that the anæsthetic employed had a very pronounced influence on the effects of sympathetic stimulation in the intact animal. Of all anæsthetics used, sodium luminal (the sodium salt of phenylethyl barbituric acid) was most striking in its effects. If the animal was anæsthetized with this drug, an inhibitor response was never obtained on sympathetic stimulation. Accordingly, further experiments were carried out in order to elucidate the action of the drug.

Method.

Cats were used throughout these experiments, since the reaction of the stomach of the dog to stimulation of the sympathetic is considerably less consistent. As a standard preparation for the comparison of sympathetic effects in the animal before and after injection of the drug we have used the spinal cat. Ether was employed, the cord being divided at the atlanto-axial joint and the brain destroyed with a probe. Artificial ventilation on oxygen was given by means of a Starling pump; the respiratory minute volume was kept low, and an adequate dead space was

provided. The thoracic sympathetic chain in the thorax was exposed, usually on the right side, by removal of two or three ribs after ligation of the intercostal vessels. The nerve trunk was freed from rami and pleura for a length of about 3 cm. up to approximately the last root of origin of the great splanchnic. The nerve was stimulated through fluid electrodes of a type slightly modified from that described by Eccles [1928]. The electrodes were filled with the defibrinated blood of the animal under experiment, and after introduction into the thorax were secured by sutures through the thoracic wall. Under these conditions the nerve retains its excitability and remains in position without interference with the thoracic wound. Stimuli were obtained from an induction coil with known current in the primary. Break shocks of a frequency varying between 1 and 70 per sec. were obtained with the contact breaker described by Brown and Lees [1931]. In order to avoid undue polarization of the electrodes, a reversing switch was placed in the secondary circuit. To record the movements of the stomach a tube was introduced through a cervical œsophagotomy after ligation of the pylorus through a small abdominal incision. The stomach was then washed out with warm saline and the tube connected to a water-float manometer, the whole system being filled with saline.

Records of the movements of the small intestine were obtained by introducing small balloons into the gut through a longitudinal incision on the antimesenteric border. The balloon was secured by a suture and the abdomen closed. The balloon was inflated and connected with a U-tube water-float manometer.

Further details of methods will be given where the technique was altered.

Experiments.

The gastric response in animals anæsthetized with luminal.

Luminal was administered to the animals in a variety of ways. It may be given orally in milk, intraperitoneally or intramuscularly without any previous anæsthetic. In many experiments the drug was injected intramuscularly in 10 p.c. solution after preliminary ether, it being possible to withdraw the ether immediately following the injection. The intravenous route may be used, but is dangerous on account of the toxic effect of the drug on the heart. We avoided giving the drug orally or intraperitoneally in view of a possible interference with the stomach movements. The anæsthetic dose is from 0·2 to 0·25 g. per kg. body weight.

In cats anæsthetized with luminal, sympathetic stimulation at all

strengths and frequencies is followed by contraction of the stomach (Fig. 1). This is, of course, quite at variance with the results obtained with the spinal cat, in which inhibition is the predominant result of sympathetic stimulation, and in which contraction can only be produced by stimulation at a frequency of 1 per sec. and occasionally with very weak stimuli.

With other anæsthetics which we have commonly used, such as ether, chloralose, and amytal, this persistent motor reaction to sympathetic

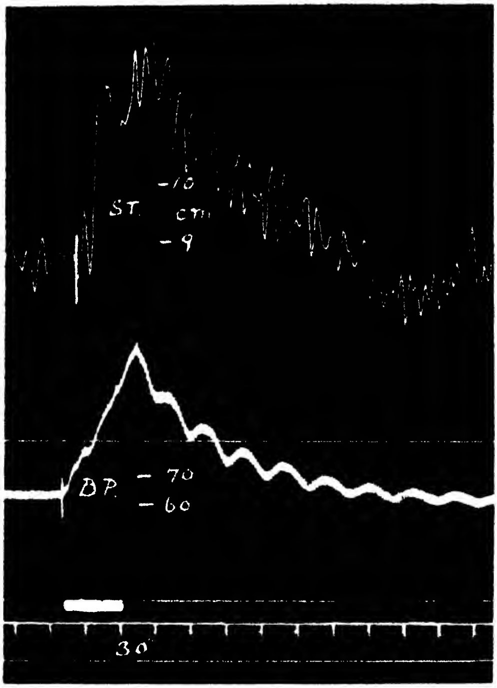

Fig. 1. Cat anæsthetized with luminal. Stimulation of right sympathetic at 60 per sec., coil 8 cm.

stimulation is not observed. With all anæsthetics considerable depression of gastric function may be observed, and, indeed, motor responses may predominate. Nevertheless, with these drugs it is almost always possible to record inhibition at some stage of the experiment, a phenomenon which is never observed under luminal.

The contraction of the stomach following sympathetic stimulation in the luminal cat differs in many details from that recorded in the spinal animal. The contraction is powerful and reaches its maximum in a few

seconds, suggesting a simultaneous contraction of the whole stomach. One striking feature is the persistence of the contraction long after the cessation of the stimulus. A stimulus of a duration of 22 sec. in one experiment was followed by a contraction of the stomach lasting 4 min.

In the spinal animal, contraction usually develops slowly, is seldom powerful, and does not greatly outlast the cessation of the stimulus. Consequently, the contraction following luminal is more nearly like a mirror image of the inhibition in the spinal cat than an exaggeration of the contraction produced by low-frequency stimulation in the spinal cat. A prolongation of the pressor effect of sympathetic stimulation is also observed. This is, however, not confined to luminal animals, as it may be seen in cats anæsthetized with amytal.

Effect of luminal in the spinal animal.

The experiments on the animal anæsthetized with luminal do not give a clear indication of the specificity of the effects of the drug, since, as previously pointed out, motor effects may predominate under other anæsthetics, and there is no method of ascertaining the characteristic response previous to the administration of the drug. Accordingly, cats were rendered spinal under ether, and the reaction of the stomach to sympathetic stimulation was determined. As previously described, the response is predominantly inhibitory, motor effects occurring rarely and only with very weak tetanizing currents or with stimuli at a frequency of 1 or 2 shocks per sec. Varying doses of luminal were then given, usually intramuscularly. The effect of an injection is shown in Fig. 2. There is complete reversal of the usual inhibitory reaction. The diminution of the rise of blood-pressure bears no relation to the loss of the inhibitory response, since, as will be shown later, these effects can be observed in the isolated preparation, and, moreover, in the animal anæsthetized with the drug the contraction of the stomach is synchronous with a very great rise in blood-pressure. This reversal of the reaction of the stomach can be obtained with a dose of the drug as low as 0·1 g. per kg. body weight, which is approximately one-half of the anæsthetic dose. If small doses are given, the reversal is not always complete, and interesting anomalous effects are occasionally observed. Stimulation of the sympathetic with a moderately strong tetanizing current caused contraction: on decreasing the strength of the current very slightly, relaxation was observed. This is the exact opposite of the occurrence in the spinal animal without luminal, in which decrease of the strength of stimulating current is either ineffective or causes contraction. The closeness of the thresholds in these

effects is remarkable—a movement of the secondary of the coil of less than 1 cm. being sufficient to change from one reaction to the other.

Another interesting series of unusual responses is sometimes observed. After luminal, stimulation is followed by a contraction which rapidly

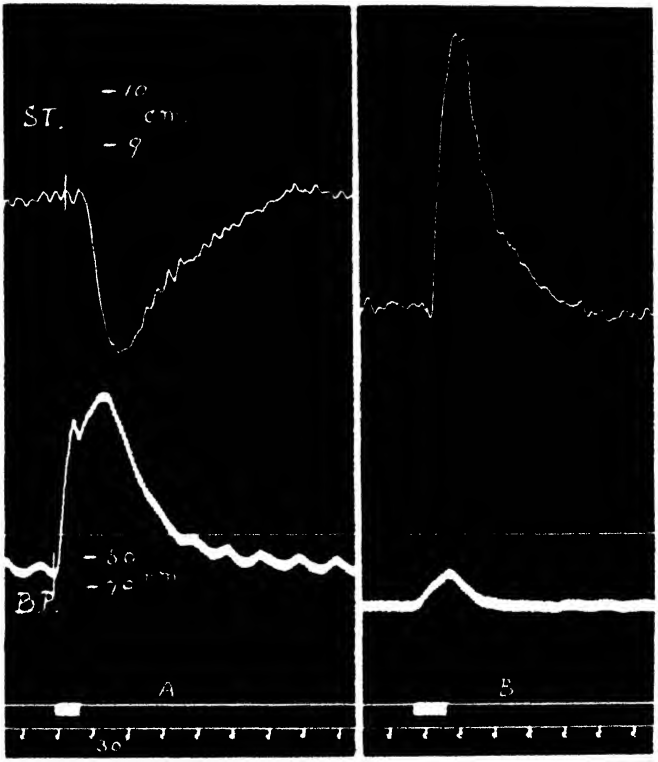

Fig. 2. Spinal cat. Stimulation of right sympathetic: A. Before luminal, 60 per sec., coil 4. B. After luminal, 60 per sec., coil 0.

passes off in spite of the continuance of the stimulation. On discontinuance of the stimulus there ensues, after a latent period of about 10 sec., a powerful and long-continued contraction (Fig. 3). This is of special interest in view of the frequency with which this phenomenon is observed in the isolated preparation.

Effect on the pyloric antrum.

It has been shown previously that the reaction of the pyloric antrum need not necessarily follow the response of the rest of the stomach to nerve stimulation [McCrea and McSwiney, 1926]. Brown, McSwiney and Wadge [1930] were unable to obtain motor responses in the

pyloric antrum of the cat, even when the body of the stomach showed contraction readily. It was therefore of considerable interest to discover whether the administration of luminal would bring out a latent motor innervation of this region.

The pyloric antrum was separated from the rest of the stomach in a number of experiments, both in the spinal animal and in the animal

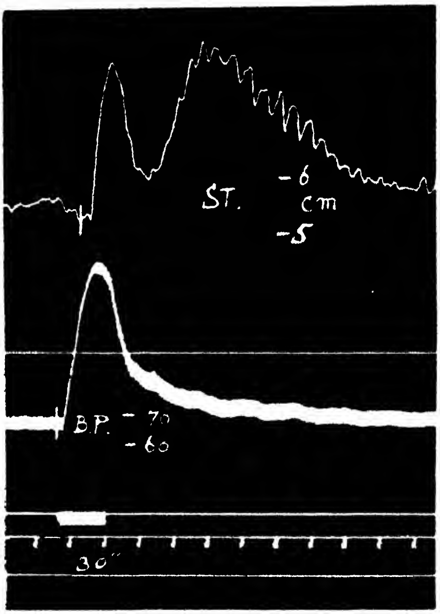

Fig. 3.

Fig. 4.

Fig. 3. Spinal cat after administration of luminal. Stimulation of right sympathetic, 60 per sec., coil 12.

Fig. 4. Spinal cat after administration of luminal. Stimulation of right sympathetic at 60 per sec., coil 4.

anæsthetized with luminal. We were, however, unable to find any clear evidence of the occurrence of motor effects. After luminal the antrum is depressed and seldom shows spontaneous movements. The only sign of a sympathetic motor effect was the occasional initiation of movements well after the termination of the stimulation. When spontaneous movements were present, the effect of sympathetic stimulation was invariably inhibitory, differing in no way from the reactions observed in the spinal cat without anæsthetic.

Effect on the small intestine.

The response of the intestine of the spinal animal to sympathetic stimulation is purely inhibitory, and the inhibitor effect persists after injection of luminal. In no experiments were we able either to reverse or even to abolish the inhibitory action of the sympathetic on the intestine. Fig. 4 shows the inhibition of the intestine persisting when the effect on the stomach was completely motor. It is of interest to note the close correspondence between the reactions of the gut and those of the pyloric antrum.

With a view to the extension of the investigation to other organs innervated by the sympathetic other than the gastro-intestinal tract, we investigated the response of the bladder in the spinal and decerebrate animal. A suprapubic urethrotomy was carried out and a cannula inserted into the neck of the bladder. Records of the bladder movements were taken by means of the usual water-float manometer. The hypogastric was isolated in the groin and stimulated. The bladder is, however, unsuitable for the study of luminal reversal, since hypogastric stimulation in the spinal and decerebrate animal results invariably in contraction. This contraction is uninfluenced by the administration of luminal in doses sufficient to reverse the gastric response. This is, in general, in agreement with the findings of MacDonald and McCrea [1930]. These observers state that relaxation of the bladder can be obtained in animals deeply under the influence of anæsthetics when the bladder is active. Luminal, in this respect, behaves in the same way as ether and chloralose.

Luminal in relation to the vagus.

It appeared to us possible that the apparent reversals of sympathetic action might be due to the effect of the drug on the relative excitability of the vagus and the sympathetic nerves supplying the stomach, one or the other becoming predominant in action. It has been shown previously that stimulation of the vagus in the neck may have one of two effects on the stomach. When the stomach is in a state of activity as a result of feeding, or of previous stimulation of the vagus, excitation of the nerve fibre in the neck is followed by relaxation. If, on the contrary, the stomach be inactive, stimulation results in contraction. Previous workers have been able to obtain both contraction and relaxation of the stomach on vagus stimulation in animals anæsthetized with luminal [McSwiney and Wadge, 1928; Veach, Schwartz and Weinstein, 1930]. In the present series of experiments we have been able to obtain similar results. In the spinal animal after administration of luminal, and in the animal

anæsthetized with luminal, we have recorded both contraction and relaxation of the stomach as a result of vagus stimulation. Contraction occurs readily, relaxation is more difficult to obtain, and activity of the stomach must be ensured by preliminary feeding and by repeated vagal stimulation. This does not, however, point to any specific action of

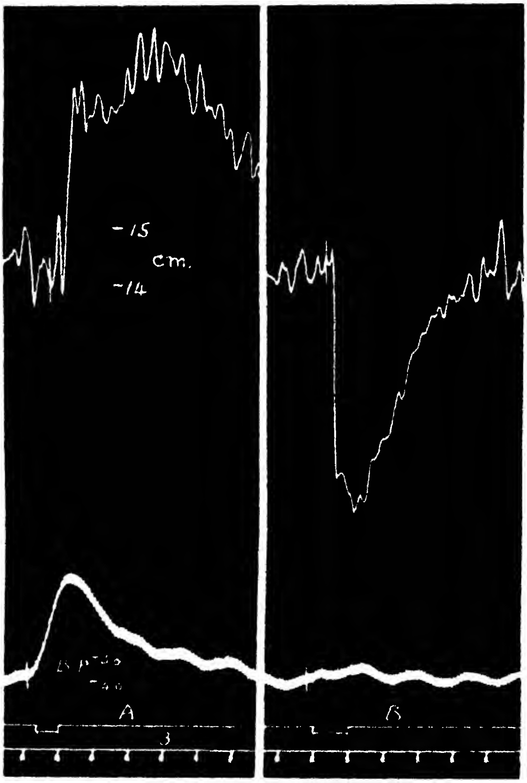

Fig. 5. Spinal cat after administration of luminal. A. Stimulation of right sympathetic, 60 per sec., coil 10. B. Stimulation of right infracardiac vagus, 10 per sec., coil 0.

luminal on the vagal system, since under many anæsthetics the same precautions have to be taken if an inhibitory effect from vagus stimulation is desired. Fig. 5 shows the inhibition due to vagus stimulation in an animal in which the response to sympathetic stimulation was entirely motor. To confirm these findings a weak solution of sodium luminal was slowly infused into a vein, and the thoracic sympathetic and the vagus were stimulated each in turn. After an interval the stomach contracted

on stimulation of the sympathetic, but a definite inhibitor response was still obtained through the vagus nerve. Later this inhibitor response was diminished, and finally only augmentor effects could be obtained with both nerves.

Effect of luminal on the response to adrenaline.

Although luminal in sufficient doses invariably abolishes the inhibitory effects of sympathetic stimulation, the effect of the drug on the reaction of the stomach to adrenaline is not certain. In the spinal and decerebrate animal the effect of adrenaline in doses of from 0·0001 mg. to 0·1 mg. is invariably one of inhibition. After administration of luminal in doses sufficient to reverse the sympathetic response, the effect of adrenaline may or may not be reversed. In the majority of experiments, no reversal of adrenaline takes place, but in a certain number of cases we observed a contraction to follow the injection of adrenaline. It may be noted that in the isolated preparation the reaction to adrenaline is never reversed by luminal.

Effect of ergotoxine.

Injection of ergotoxine in doses of 0·15 mg. per kg. abolishes the motor effect of sympathetic stimulation after luminal. In a small number of experiments complete reversal of the motor effect took place, and sympathetic stimulation resulted once more in relaxation as in the animal without luminal. This complete recovery of the inhibitory effect is not commonly seen, and the inhibition when observed is of small magnitude.

Effect of luminal on the isolated innervated strip.

The experiments described, while demonstrating that injection of sodium luminal abolishes the inhibitory effect on the stomach of stimulation of the sympathetic, leaving unimpaired the motor response, do not, however, afford evidence indicating the locus of action of the drug. In the intact animal, many factors, *i.e.* alterations in the vascular conditions, the action of the drug on the gastric muscle, interference with the sympathetic reflex arcs, may all predispose to the predominance of a motor response to sympathetic stimulation.

An attempt was therefore made further to localize the action of the drug by stimulating the postganglionic fibres of the sympathetic supply of the stomach. The animal was prepared in the usual way, and then the splanchnic and the semilunar ganglion were exposed through a lumbar incision. The experiment, however, was unsuccessful for two reasons:

first, the high threshold of the postganglionic fibres necessitated stimulating currents of such magnitude that it was practically impossible to avoid very extensive spread of current, and, secondly, because the postganglionic fibres are distributed in such a diffuse manner from the ganglion that stimulation of any one group would result in movement of so small a region of the stomach that it would be impossible to record.

Accordingly, strips of the stomach retaining their peri-arterial nerve supply were prepared according to the technique of McSwiney and Robson [1931*a*]. Stimulation of the peri-arterial nerves in these preparations results in either contraction or relaxation of the muscle, according to the type of stimulation employed. Stimulation at a frequency of from 1 to 3 per sec. usually causes contraction, whereas when stimuli are applied at a frequency of 20 per sec. or over, relaxation is the usual response. McSwiney and Robson [1931*a* and *b*] have brought forward evidence to show that the effects produced are distinct from the results of vagus stimulation. In our experiments, a slight modification of the technique was instituted in that the strips were taken, not from animals killed under ether, but from decerebrate animals which had been allowed to ventilate off their anæsthetic. We were able to confirm their previous findings. The addition of luminal to the bath containing the preparations reproduced almost exactly the effect of injecting the drug into the intact animal. After giving luminal in doses sufficient to bring the concentration in the bath to between 1:70,000 and 1:20,000, the inhibitory effect of sympathetic stimulation disappears. Fig. 6 shows the inhibition obtained before addition of the drug; afterwards all types of stimulation produced only contraction. Since, in all these preparations, there is a tendency for the inhibitory effects of the sympathetic to disappear as the preparation deteriorates, it is noteworthy that we were able to recover the inhibitory effect by washing out the luminal by means of a single change of the Ringer solution of the bath.

Complete reversal of the inhibitory effect does not occur immediately, but takes some 20–30 min. to develop, and in some preparations it is possible to observe the gradual onset of the change. The inhibitory effect of stimulation becomes less and less until a point is reached at which stimulation has no effect. Following this, the motor effect gradually develops and reaches a maximum (Fig. 6).

Anomalous effects due to incomplete reversal were observed, which resembled very closely those obtained in the intact animal. In the strip untreated with luminal, the relaxation following stimulation is frequently succeeded by a small "after-discharge" contraction which follows the

cessation of the stimulation after a latent period of about 3·0 sec. When luminal is given in doses which are sufficient to produce a complete reversal, the appearance of the after-discharge contraction in a strip which did not previously show it may be the only sign of the action of the drug. The following steps are sometimes observed in the development of the reversal: the untreated strip shows an uncomplicated inhibition, after luminal the inhibition is followed by an after-contraction, the inhibition gradually diminishes and the after-contraction increases; a stage of no

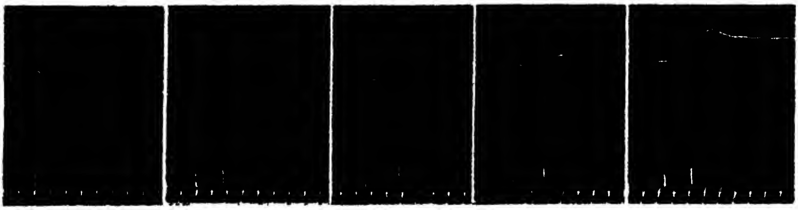

Fig. 6. Stimulation of peri-arterial nerves of isolated stomach strip, at 25 per sec., coil 5; time 10 sec. Development of reversal after addition of luminal.

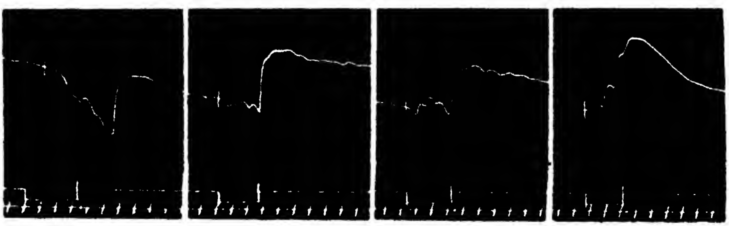

Fig. 7. Stimulation of peri-arterial nerves of isolated stomach strip, at 50 per sec., coil 3; time 10 sec. First stimulation, before luminal. Development of reversal and after-discharge contraction.

effect is reached in which the actual stimulation has no visible effect, but cessation of the stimulus is followed by a large contraction; the next stage is the appearance of a small contraction during the stimulation followed by the after-contraction, and, finally, the stimulus contraction becomes maximal and fuses with the after-contraction giving a large and apparently homogeneous motor effect (Fig. 7). Some measurements of latent periods were made. The latency of relaxation before and after luminal was of the order of 5 sec., a figure which agrees well with those found by McSwiney and Robson. The latency of the contraction after luminal appeared to be slightly increased beyond normal.

The response to adrenaline of the isolated strips is of interest. Luminal does not reverse the inhibitory effect of the drug in any concentration, adrenaline inhibition persisting when the sympathetic response is purely motor.

We also investigated the effect of luminal on the reactions of the cat's isolated intestinal segment retaining its innervation through the peri-arterial nerves. Luminal, as in the intact gut, has no effect on the inhibition resulting from peri-arterial stimulation. We are aware that other observers have obtained contraction on stimulation of the peri-arterial nerves, but these results were obtained using a preparation of the intestine of the rabbit.

Discussion.

These experiments show that luminal abolishes the inhibitory effects of sympathetic stimulation on the stomach. The similarity between these effects and those of ergotoxine on the circulatory system is apparent. With luminal, however, the reversal is in the opposite direction, an inhibition being converted into a contraction. A striking feature of the action of the drug is its extreme specificity, the action being confined, as far as the present investigation shows, to the stomach.

Before the action of the drug is considered in detail, attention may be drawn to two points. First, that the inhibition and contraction resulting from sympathetic stimulation is not in any way dependent upon vaso-constriction, as was suggested by Veach [1925] and others. For, not only does inhibition occur readily in the isolated preparation, but contraction of the stomach is observed in the intact animal after luminal, when the rise in blood-pressure is as great as when inhibition occurred before the administration of the drug. Secondly, the effectiveness of luminal in the isolated preparation demonstrates clearly that the drug acts, at least, peripheral to the semilunar ganglion, and its action is presumably on the peripheral neuromuscular mechanism.

The phenomenon of limitation of the action of luminal to that part of the alimentary canal which can, in certain circumstances, give a motor response to sympathetic stimulation (*i.e.* to the body of the stomach) and its absence of effect on those parts which do not in any condition give contraction (*i.e.* the antrum and the small intestine) may suggest that the drug does not cause a true reversal, but allows the action of motor fibres to become apparent by paralysing the inhibitors. This hypothesis, however, does not bear critical analysis, since after luminal, inhibition persists unaltered in the antrum and the small intestine. Moreover, as

previously stated, the contraction of the intact stomach after luminal is remarkably like a mirror image of the inhibition obtained before luminal. This becomes more clear if the relation between the duration of stimulus and response be studied in more detail. We have measured the duration of stimulus and the duration of motor response and inhibitor response respectively, before and after the administration of luminal. The mean of thirteen measurements gave a value of 1·46 for the ratio

$$\frac{\text{Duration of motor response}}{\text{Duration of stimulus}}$$

before luminal. This figure shows how closely the contraction follows the duration of the stimulus in the normal animal. After luminal the mean of twenty-two observations of the same ratio was 5·85. This is remarkably like the figure obtained from $\frac{\text{Duration of inhibition}}{\text{Duration of stimulus}}$ in the animal without luminal which was 6·5 (twenty-two observations).

The supposition that the phenomenon is one of true reversal rather than abolition of one set of fibres and the over-action of another becomes more valid if the results of the experiments on the isolated innervated preparation be considered. Frequently in these experiments, after the administration of luminal, it is possible to watch the gradual diminution of the relaxation until nerve stimulation is without effect. After this "null point" it is possible to observe the gradual development of the contraction (Fig. 6).

There is then some evidence that luminal acts by virtue of a peripheral reversal, but only in those tissues which in normal conditions can show a twofold response to sympathetic stimulation. In recent papers by Kuré and others [1930, 1931], a set of fibres is described, running in the sympathetic, which, on account of their origin in the cord, are regarded as spinal parasympathetic. Criticism of the histological evidence brought forward by these authors has been made in another place [McSwiney, 1931]. These observers further suggest that the motor effects of sympathetic stimulation are due to the presence of these fibres, the true sympathetic being purely inhibitory in its action. Their evidence is based on the direct observation of the stomach and gut following the section and stimulation of nerves and the painting of the semilunar ganglion with nicotine. It is possible that the vagus and sympathetic supply of the stomach is composed of two sets of fibres, and, indeed, we have previously postulated the presence of motor and inhibitor fibres in the sympathetic chain [Brown, McSwiney and Wadge, 1930]. We cannot, however, agree that the results obtained by us in these and in previous experiments can be explained in this simple

manner. Should the contention of Kuré be true, the reversal by luminal would be explicable on the assumption that it paralysed the true sympathetic. It seems, however, as stated above, inconceivable that the drug should be so specific in its action as to be able completely to paralyse the gastric (inhibitory) sympathetic while leaving unaffected the sympathetic supply, not only of the small intestine, but also of one part of the stomach itself, namely the pyloric antrum. The fact that the contraction following sympathetic stimulation is abolished by ergotoxine is again in favour of the contraction being of sympathetic rather than parasympathetic origin. Collateral evidence, which although not strictly relevant is nevertheless significant, is the fact that McSwiney and Robson [1931*b*] have shown, not only that the contraction following sympathetic stimulation has a very different latent period from that following vagus stimulation, but also that sympathetic contraction has actually an inhibitory effect upon any subsequently induced vagus contraction. Furthermore, Brown and Garry [1931] have shown that the inhibitory effects of vagus stimulation on the stomach are removed by administration of amytal without greatly affecting the sympathetic inhibitory and motor effects.

We may state then that the contraction which follows stimulation both of the preganglionic and postganglionic sympathetic supply of the stomach is probably due to stimulation of true sympathetic fibres. The question as to whether the two effects are due to the stimulation of specific motor and inhibitor nerves or to the differential peripheral action of one physiologically homogeneous nerve is more difficult. The evidence given above appears to show that the action of luminal is one of true peripheral reversal, which occurs only in those tissues which can normally show a dual response to sympathetic stimulation. This suggests that the normal duality of response is rather of the nature of a peripheral effect than due to the presence of two sets of nerve fibres which are set into action by different types of stimulation. There exists at present a considerable body of evidence in favour of the hypothesis that the sympathetic acts by the liberation in the periphery of some specific substance. Presuming that the nerve is functionally homogeneous, two possibilities present themselves: first, that the nerve can liberate an excitor or an inhibitor substance according to the type of stimulation or to the condition at the time of stimulation; secondly, that the nerve liberates only one substance, the effect of which can be either excitor or inhibitor according to various conditions at the periphery.

With regard to the second suggestion, there is no doubt that sympatho-

and parasympathomimetic drugs can in certain conditions elicit both excitor and inhibitor responses from the muscle of the stomach. Brown and McSwiney [1926] and McSwiney and Brown [1926] were able to obtain reversal of the action of adrenaline on isolated strips both of the stomach and the uterus by the previous administration of excitant or depressant drugs. McCrea and MacDonald [1929] have shown that sympathetic and parasympathetic drugs may have excitor or inhibitor effects according to the state of activity of the stomach; the effects of atropine on the gut before and after the removal of choline [Le Heux, 1920] fall into the same category. There appears, therefore, to exist ample evidence that certain drugs of the autonomic class can exert a dual action on plain muscle. Assuming that the humoral theory of the action of these nerves be accepted, the duality of action of autonomic drugs absolves one from the necessity of postulating the presence of four liberated substances to account for the dual action of both the vagus and the sympathetic. One feature is common to the experiments quoted above concerning the reversal of the effects of autonomic drugs, namely depression of the tissue is the usual means of revealing the latent excitor effect of a normally inhibiting substance, and, conversely, excitation can reveal the inhibitory action of a normally excitant substance. Similar effects are to be observed in the effects of nerve stimulation.

Reverting now to the sympathetic innervation of the stomach, contraction. instead of the more usual relaxation, occurs in three conditions (*a*) when the sympathetic chain is stimulated at a low frequency, (*b*) after the administration of luminal, and (*c*) when the activity of the stomach is materially depressed [McCrea and McSwiney, 1928]. This association of low-frequency stimulation, depression of activity and the effectiveness of a drug which is a powerful general anæsthetic suggests strongly that luminal acts by depressing either the rate of formation, the amount formed or the activity on the cell of the hypothetical sympathetic substance. That it is the amount formed or the rate of formation which is largely affected is favoured by the fact that the action of adrenaline is reversed but seldom in the intact animal and never in the isolated preparation.

Accordingly, with the information at present at our disposal we feel justified in suggesting the following as explaining the majority of the observed phenomena, namely that the sympathetic acts by liberating a specific substance which has either an augmentor or an inhibitor effect upon the stomach muscle. The direction of its action appears to depend upon its rate of liberation or action upon the tissue, luminal acting by depressing the rate of production or action.

Summary.

Experiments were carried out to show that sodium luminal (phenylethyl barbituric acid) abolishes the inhibitor effects on the stomach of stimulation of the thoracic sympathetic chain. After anæsthetizing with luminal or injecting the drug into the spinal cat, the inhibition is converted into contraction. Though sympathetic stimulation after luminal causes augmentor effects, relaxation is still obtained with the vagus nerve. Luminal does not reverse the response normally obtained with the sympathetic from the pyloric antrum and small intestine. The action of luminal is claimed to be peripheral, as reversal was demonstrated using the isolated nerve-gastric muscle preparation. The adrenaline response may be reversed in the spinal animal after luminal, but this does not occur in isolated preparations. From the evidence it is suggested that the sympathetic acts by liberating a specific substance which has either an excitatory or inhibitory action upon the stomach muscle, the direction of its action depending upon the rate of liberation or action upon the tissue. Luminal acts by depressing the rate of production or action.

We wish to express our thanks to Miss W. J. Wadge for her assistance in the early part of this investigation.

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PHOSPHATE IN TUMOURS AND MUSCLE.

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THE fermentation of sugar is a fruitful source of anoxidative energy in muscle, yeast and tumours. Phosphate is concerned in muscle and yeast glycolysis as follows:

(1) Fermentable compounds of hexoses with one and with two molecules of phosphate have been isolated from both.

(2) Inorganic phosphate and adenylpyrophosphate form part of the coferment system necessary for the glycolysis of glycogen by muscle extract, the latter compound also serving in the coferment of yeast [Lohmann, 1931].

(3) Creatine-phosphate is possibly related to this system since the anaerobic breakdown of the pyrophosphate fraction in muscle does not take place until the creatine-phosphate has been exhausted [Eggleton, G. P. and P., 1929].

The question naturally arose whether glycolysis of tumours involved phosphate as in muscle and yeast. Barr, Ronzoni and Glaser [1928] found only a small liberation of free phosphate during glycolysis by surviving tumour, and the addition of phosphate affected neither lactic acid production nor sugar disappearance. Lange and Henning [1928] found that the free phosphate washed out of tumours, surviving in Ringer fluid containing glucose, did not parallel the production of lactic acid.

There is certain indirect evidence in favour of phosphate intermediaries in surviving tumour glycolysis. Lactic acid formation by tumour is inhibited by fluoride [Ewig, 1929; Dickens and Simer, 1929], by amylase [Harrison and Mellanby, 1930], and by monoiodoacetic acid [Harrison and Mellanby, 1931]; in muscle these substances inhibit lactic acid formation by either preventing the synthesis or breakdown of hexosephosphate esters. Neuberg, etc. [1930] found that the residue from Jensen's rat sarcoma, after acetone extraction, formed some free phosphate and methyl glyoxal from magnesium hexosediphosphate. Since Warburg and others [1924] have shown that intact

tumour cells can rapidly form lactic acid from methyl glyoxal, these authors inferred that the lactic acid formation in tumour follows a similar path to that of other glycolytic systems. On the other hand, Harrison and Mellanby [1930] found that surviving mouse carcinoma could not produce lactic acid from added hexosemonophosphate or hexosediphosphates. However, Downes [1929] reported some glycolysis of a "hexosephosphate" by rat sarcoma.

This paper reports further investigation into the possible rôle of phosphate in tumour glycolysis as compared with muscle.

METHOD.

Crocker Institute 180 mouse tumour was used throughout. Changes on glycolysis in the surviving tissue were followed by comparing the extracts of alternate slices before and after 1 hour's incubation at 37·5° C. From three to six mice supplied between 1 and 2 g. for each of the control and incubated tissue. Slicing of each tumour was complete within about 8 minutes after the death of the mouse. The control slices were immediately placed in ice-cold 5 p.c. trichloroacetic acid solution. The slices for incubation were put in 50 c.c. of isotonic Ringer solution containing 0·015 *M* sodium bicarbonate. The fluid was kept in a Petri dish and continually agitated during incubation. The gas phase was a 5 p.c. solution of CO_2 in air. Aseptic precautions were taken throughout and after incubation were checked by cultures. The tissues, after grinding, were left in the ice chest to extract overnight in 5 p.c. trichloroacetic acid, then centrifuged.

After treating an aliquot part with lime and $CuSO_4$, the lactic acid was measured directly by the Friedemann and Kendall [1929] method using $MnSO_4$ and $KMnO_4$. Phosphorus was measured by Martland and Robison's modification [1926] of the Briggs method.

Considerable difficulty was encountered in preparing a clear trichloroacetic acid extract of tumour tissue. The method finally adopted was to neutralize the acid extract with Na_2CO_3, finally adjusting to *p*H 7·0 by drawing off CO_2. A further slight precipitate was centrifuged down, but even this process did not yield perfectly clear extracts.

EXPERIMENTAL.

Labile phosphate in tumour extracts.

To avoid confusion in the free phosphate estimation of the tumour extracts it was first necessary to investigate the incidence of labile

phosphate. It soon became apparent that there was little, if any, phosphate present that was rapidly hydrolysed by acid. The time curve for the development of colour of the phosphate reading, as compared with standard, was practically a straight line only slightly rising within 40 minutes. This was with extracts from tumour tissue removed from the body within 3 minutes of death and immediately ground in ice-cold trichloroacetic acid solution.

To investigate this slight rise further, the more recent method of Eggleton and Eggleton [1929] for creatine-phosphate was used. The results of four experiments on the tumour are given in Table A. Two

TABLE A. Creatine-phosphate in C 180 mouse tumour.

Experiment	Tissue	Weight g.	"Creatine" P Observed mg.	"Creatine" P mg. per 100 g.	Ba-"soluble ester" mg. per 100 g.	Free P mg. per 100 g.	"Pyro" P mg. per 100 g.	Ba-"insoluble ester" mg. per 100 g.
350	C 180 mouse tumour	1·40	0·0037	1·5	13·3	32	5·4	11·1
351	,,	1·23	0·0036	2·0	15·5	15·5	7·6	7·9
360	,,	0·87	0·0041	2·3	5·5	29	2·5	21·2
361	,,	0·67	0·0039	2·7	3·8	30	3·5	18·1
362	Mouse leg muscle	1·00	0·0467	22·5	20·5	58	34	14
363	,,	1·55	0·0615	19·5	18	72·5	39	18

experiments done on mouse leg muscle are given as controls. It will be seen that there is only a small quantity, if any, of a substance with the solubility and lability similar to creatine-phosphate present in C 180 tumour. The observed readings are very near to a blank. There is a definite though variable amount of barium "soluble ester," and barium "insoluble ester" apart from that hydrolysed as pyrophosphate. The "pyrophosphate" recorded is simply the increase in reacting phosphate of the barium insoluble fraction after 7 minutes' boiling in *N* HCl. As Boyland [1930] points out, this would include about one-third of the phosphate of any Harden and Young hexosediphosphate present. Both the pyro- and creatine-phosphate are present in about the same concentration as Eggleton and Eggleton found for testis and uterus.

It is therefore not surprising that little free phosphate increase has been found on incubation of the mouse tumour. However, there are present fractions which correspond to the chief hexose esters so far described from muscle and yeast. The barium soluble "ester" would contain any hexosemonophosphate present, whereas most of any hexosediphosphate would be included in the insoluble residue. Although the tumour extracts contained only negligible quantities of labile phosphorus, it was

possible that intermediary phosphate shifts were catalysed by the acid in the extract. All extracts were therefore kept cold until neutralized.

Phosphate balance in incubated mouse tumour.

Incubation experiments were done on the C 180 mouse tumour with and without adding 0·2 p.c. glucose to the Ringer fluid. By comparing the two it was hoped that any excess synthesis or breakdown of a phosphate intermediary might be demonstrated.

Since the incubation tissue in the Ringer fluid was difficult to weigh, we assumed its weight bore the same ratio to its total phosphate content as the control. The control tissue was weighed and the weight of the incubated tissue computed from its relative phosphate content. On checking this method by weighing and estimating the total phosphate of both the incubated and control, we found the computed and observed results from the alternate slices did not vary by more than 5 p.c. In fact the phosphate per unit wet weight of different tumours did not vary by more than twice this amount.

The neutralized trichloroacetic acid extracts were both made up to 72 c.c. with distilled water and divided into 14 equal parts of 5 c.c. each. On one, the free phosphate was determined, on another the lactic acid, and two more served for duplicate total phosphate estimations after digestion. The total phosphate was also done on the sodium carbonate and trichloroacetic acid precipitates. From the sum of these three total phosphates, the total phosphorus of the tissues was obtained.

In Table B the changes on 1 hour's incubation expressed as mg. per 100 g. wet weight are given. In incubation with added sugar the average free phosphate of six experiments increases by about 5 mg. per 100 g., an amount in excess of any labile phosphate present. For seven lots of tissue incubated without sugar the average free phosphate liberated is over twice this amount. Thus the free phosphate liberated during incubation is greater in the absence of added sugar than when sugar is present. Interpretation of these results is complicated by the fact that as shown in column 2, during incubation of the tumour in Ringer solution, there is also an increase in the total acid soluble phosphate. Although variable in different experiments, this increase averages about the same whether sugar is present or absent in the fluid. This would suggest the increase results from the breakdown of the cells damaged in slicing. The net result of these changes is that there is an increase in the "organic" phosphate during incubation in the presence of sugar which does not occur when sugar is absent.

TABLE B. Changes on incubation of C 130 tumour with and without added glucose (mg. per 100 g. wet weight).

Mouse tumour experiment	Glucose in fluid	(1) Free P increase	(2) Total acid-soluble P increase	(3) "Organic" P increase (2)−(1)	(4) "Organic" P increase from precipitation curve 20 to 70 p.c.	(5) Difference between (3) and (4)	(6) Lactic acid formation	Culture of incubation fluid
252	+	1·7	4·1	2·4	—	—	460	?
304	+	8	16·2	8·2	7·5	0·7	430	Slight growth
377	+	4	15·6	11·6	11·4	0·2	—	No growth
385	+	7	11·4	4·4	3·3	1·1	640	Slight growth
389	+	3	10·1	7·1	7·8	0·7	720	,,
403	+	2·5	12·8	10·3	8·4	1·9	700	No growth
	Av.	4·4	11·7	7·3				
321	0	9·5	6·4	−3·1	—	—	30	No growth
341	0	4·5	4·9	0·4	0·6	0·2	25	?
359	0	9·0	5·6	3·4	−1·5	1·9	—	Several colonies
367	0	9·5	7·4	−2·1	−1·3	0·8	40	Slight growth
372	0	14·5	11·5	−3·0	−1·1	1·9	30	,,
382	0	22·2	19·6	−2·6	−4·0	1·4	70	No growth
392	0	16·9	16·9	0	—	—	40	Slight growth
	Av.	12·3	10·3	−2				

Average, Free P 21·7, Total acid-soluble P 53·4, Total P 238.

The above results represent merely the balance of all the phosphate changes that occur during incubation. If glycolysis of the tumour does involve phosphate intermediaries, in the absence of added sugar the 2 or 3 mg. of free P produced in excess of the new acid soluble P could not account for the appearance of some 30 mg. of lactic acid from the breakdown of any known hexosephosphate. Either the tumour was able to utilize sugar remaining in the cell or there had been a shift from one type of ester to another, as for example, from a mono- to a di-phosphate form with the liberation of a hexose molecule for glycolysis, but no free phosphate. Further experiments were made to test this point.

Fractional precipitation of barium salts by alcohol.

In view of the variation in properties and function of the various phosphates already isolated from other glycolysing tissues, it was essential that any method adopted for following similar substances in tumour should be as general as possible. The method used depends upon the fact that the barium salts of most of the phosphate compounds isolated from muscle and yeast have fairly characteristic solubilities in different concentrations of alcohol. Thus Ba-hexosediphosphate is insoluble in 10 p.c. alcohol at about pH 8, whereas Ba-hexosemonophosphate is soluble in

that concentration but is precipitated by 70 p.c. alcohol from a solution of the same acidity. Likewise Ba-pyrophosphate is insoluble in water whereas the barium salts of creatine-phosphate and adenylic acid are soluble at *p*H 8, that of the latter being precipitated by four volumes of alcohol.

Since it was not known that the phosphates in tumour were similar to any of the above, we adopted the more general plan of estimating the "organic" P precipitated by ten different concentrations of alcohol from 0 to 85 p.c., in the presence of barium. The results were plotted as a curve with the alcohol concentration as the abscissa. By comparing the levels and shape of these curves before and after incubation, the synthesis, or shift from one type of phosphate to another with different solubility, could be detected. In an attempt to determine the curve at a *p*H which would differentiate as many substances as possible, the extracts were brought to *p*H 7.

Fractional precipitation of barium compounds by alcohol was therefore done on the remaining ten aliquot parts of the trichloroacetic acid extracts from the experiments quoted in Table B. To each 5 c.c., four drops of saturated $BaCl_2$ solution were added (about 0·1 g.). A different amount of ethyl alcohol was then added to each to make ten different concentrations from 0 to 85 p.c. by volume. These were left to precipitate overnight in the ice chest, then centrifuged. The clear supernatant fluids were decanted into another set of tubes, evaporated to dryness, and the total P estimated after digestion. By subtracting this value from the total acid soluble P of the duplicate 5 c.c. quantities, the total P of the precipitate from each alcohol fraction was obtained. To obtain the free P, the Ba precipitates were taken up in 5 c.c. of 0·1 *N* acetic acid, 1 c.c. of 10 p.c. sodium sulphate added and the barium sulphate centrifuged off. The free phosphate was then estimated in the supernatant fluids. This procedure yielded a 96 p.c. recovery from an orthophosphate solution of 0·04 mg. P content. The "organic" phosphate precipitated by each alcohol fraction was then found by the difference between the free and the total phosphate. This figure would of course contain such pyrophosphate as was present. Four experiments were done in which the total P was estimated on both the precipitate and the supernatant fluid of each alcohol concentration. Unless grossly contaminated, their sum agreed within the same range as that of the duplicate situations of total acid-soluble P, *i.e.* about 0·002 mg. P.

Typical fractional precipitation curves of experiments on mouse tumour are given in Fig. 1. These curves are for the "organic" phosphate precipitated from the extract before and after incubation and the

results are expressed in mg. P per 100 g. wet weight of tissue. In the case of the tumour incubated with sugar it is seen that the increase in the acid-soluble "organic" phosphate is due to one fraction which is precipitated completely by all alcohol concentrations from about 20 p.c.

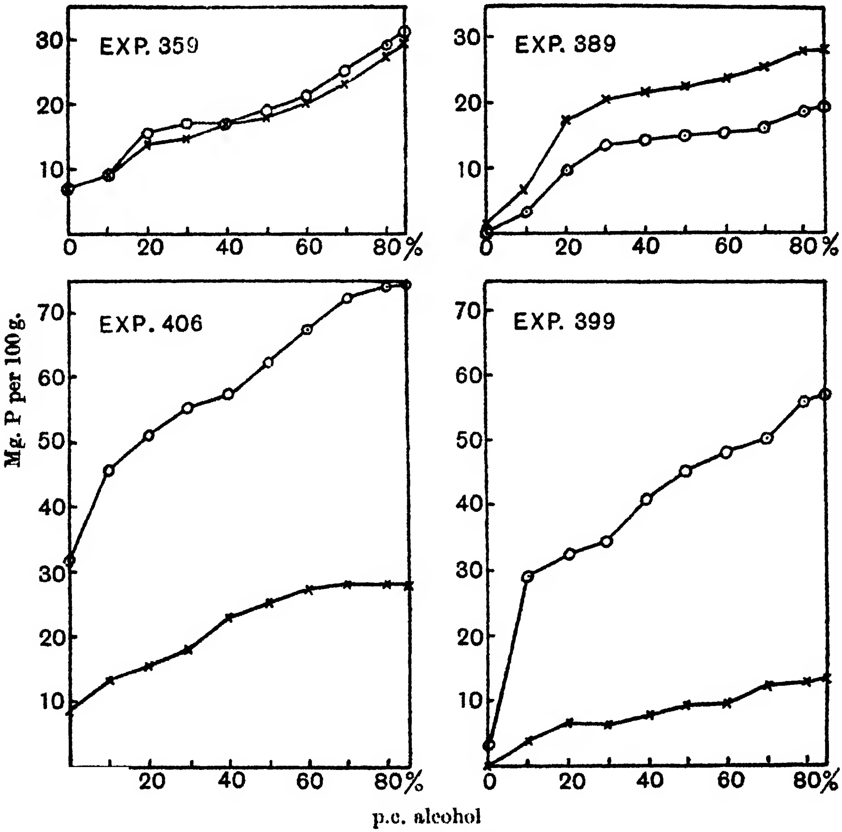

Fig. 1. Fractional barium alcohol precipitation curves for acid-soluble "organic" P expressed in mg. per 100 g. wet weight. Two upper curves are for sliced C 180 mouse tumour. Two lower for minced mouse leg muscle. In two left curves no glucose added to Ringer fluid (0·015 M $NaHCO_3$). Two right curves fluid contained 0·2 p.c. glucose. Control (before incubation), 0–0, after incubation (1 hour) x–x.

up. Apart from this increase the curves are practically parallel. The curves for the tumour incubated without added sugar are parallel and practically coincident. There is no evidence of any increase in the organic P precipitated at 20 p.c.

Since the curves are parallel from 20 p.c. up in tissue incubated with

and without sugar there cannot have been any uncompensated shift from one organic phosphate formed to another in this range: that is, there cannot have been any shift from a hexosemonophosphate to a hexosediphosphate during the incubation for 1 hour, of the C 180 tumour with or without the addition of sugar to the fluid. The accuracy of this statement can be seen from Table B in column 4 of which is tabulated the average change in the organic P precipitated by 20 to 70 p.c. alcohol inclusive. It is seen that this figure varies by less than 2 mg. per 100 g. from the difference between the increase in the acid-soluble P and the increase in the free P as given in column 3. In other words, all the phosphate changes can be accounted for within less than 2 mg. per 100 g. by a free P increase and an "organic" P increase at 20 p.c. alcohol concentration.

Since this organic fraction is all precipitated by 20 p.c. alcohol it behaves as a single substance. Moreover, the increase in free P in the absence of added sugar is either from the breakdown or non-synthesis of a substance having the same property, since not only does the 20 p.c. rise not appear in the curves but there is no other significant change in them to account for the free P balance as given in Table B. If this "organic" substance is a part of the new acid-soluble P derived from the incomplete breakdown of the acid precipitate, then, in the absence of sugar when the energy from glycolysis is much reduced, it is completely broken down to liberate its phosphate; or if it is the result of synthesis, then this synthesis does not take place in the absence of added sugar. It will be noted that this substance has a solubility in keeping with a hexosediphosphate.

Since the increase in the acid-soluble P was at the expense of substances precipitable by trichloroacetic acid, it was of some interest to follow the ether-soluble P. The average ether-soluble P of ten lots of C 180 tumour was 4·7 mg. per 100 g. As the result of 1 hour's incubation this was increased by 3·4 mg. This increase was not significantly affected by the addition of sugar. Mouse muscle under similar conditions showed no change. There is therefore a decrease in the acid-precipitable non-lipoid P of tumour during incubation.

Phosphate in incubated minced muscle.

As a check on the method used in obtaining the above precipitation curves, and by way of comparison with tumour, similar experiments with mouse muscle were made. The animals were killed by stunning as before, and for the control, the muscle of one hind limb quickly removed

and placed in a tared weighing bottle containing 10 c.c. of 5 p.c. trichloro-acetic acid solution in an ice mixture. The muscle of the other hind limb, after mincing finely with scissors, was used for the incubated tissue. Aseptic precautions were taken throughout and cultures were invariably sterile. Apart from the use of acid washed sand in grinding, the technique was as previously described for tumour.

In estimating the free P of the precipitates, readings were made about 40 minutes after adding the sulphuric acid and other ingredients. The results therefore included any labile P present in the precipitates. The soluble Ba-creatine-phosphate was absent from the lower alcohol precipitates. This had to be allowed for. The "organic" values thus obtained correspond to those of the mouse tumour where the labile P was practically negligible, and the free P curves similar to those of Ba-orthophosphate of incubated muscle, *i.e.* maximal at about 30 p.c. alcohol.

The total phosphates and lactic acid for the incubation experiments done on minced mouse muscle are given in Table C. It is seen that the

TABLE C. Incubated minced mouse leg muscle in bicarbonate buffered Ringer's solution with and without glucose.

Experiment	1 hour incubation at 37·5° C.	Tissue weight	Glucose in fluid (0·2 p.c.)	Free P plus labile P: Mg. per 100 g.	Free P plus labile P: Increase	Total acid-soluble P: Mg. per 100 g.	Total acid-soluble P: Change	Lactic acid: Mg. per 100 g.	Lactic acid: Increase	Total P of tissue mg. per 100 g.
399	Control	1·47		88		150		160		
	Incubated	1·68	+	131	43	149	−1	430	270	228
417	Control	0·81		71		129		220		
	Incubated	0·78	+	115	44	134	+5	510	290	237
406	Control	1·50		92		170		160		
	Incubated	1·34	0	153	61	172	+2	270	110	269
409	Control	1·70		80		155		130		
	Incubated	1·46	0	133	53	158	+3	290	160	245

lactic acid produced by the muscle pulp in the absence of added sugar is much greater than with the tumour. The large free P increase (apart from the creatine-phosphate) without any significant increase in the total acid-soluble P is noted in contrast to tumour. This decrease in the "organic" P is reflected in the alcohol precipitation curves. Typical barium fractional curves for the minced mouse muscle before and after incubation are shown in Fig. 1. The results as before are expressed as mg. P per 100 g. wet weight. It is seen that the upper control curves show a definite break at about 30 p.c. alcohol, indicating a fraction completely precipitated at about this concentration. There is also some evidence,

particularly in experiment 399, of another break occurring in the neighbourhood of 70 p.c. alcohol. This was quite marked in three of the five experiments done, indicating a complex nature for the more-soluble fraction.

The lower curves represent the same "organic" fraction after incubation. There is no significant change noticed due to the addition of glucose in contrast to tumour. It is also evident that the increase in free P during incubation (apart from the creatine P) is derived from at least part of both the more-soluble and the less-soluble fractions. By comparing the acid hydrolysis of extracts of rabbit muscle before and after incubation Davenport and Sacks [1929] likewise found this "enzyme hydrolysable" fraction behaved as at least two different substances. It is not possible from Ba precipitation experiments to deduce the exact amount of free P contributed by either fraction. The liberation of (more-soluble Ba-) adenylic acid from (less-soluble Ba-) adenylpyrophosphate (Lohmann) would involve an increase in the more-soluble fraction with a decrease in the less-soluble, without, *per se*, involving any liberation of free P. In Eggleton's experiment with oxygen lack on muscle a time relationship is noted between the disappearance of the pyro-P and "insoluble" ester, and the increase in the "soluble" ester. In the minced muscle there is definitely some liberation of free P from the pre-existing more-soluble fraction.

Discussion.

From these results on C 180 mouse tumour and minced muscle certain comparisons may be made. The lactic acid produced in 1 hour by minced muscle without sugar is much larger than that produced by the surviving tumour under similar conditions. Several authors are agreed that tumour contains a comparatively large quantity of glycogen. Presumably this cannot undergo glycolysis to the same extent as in muscle.

The free P of the tumour is of the same order as that found for resting muscle which is in diffusible equilibrium with a free P concentration similar to that in plasma. In contrast to the muscle there is only a very small quantity, if any, of labile P present in the tumour. By comparing the fractional precipitation curves it is evident that the "organic" acid-soluble P which exists in comparatively small quantities in tumour does not behave similarly to that of minced muscle during incubation. By comparing the behaviour of the tumour incubated in the presence and absence of added glucose it is seen that the liberation

of free P bears a certain reciprocal relation to the formation of lactic acid and in this respect behaves similarly to the liberation of ammonia as shown by Warburg.

While this paper was being reported, further work on phosphate metabolism of tumour has been published by Edlbacher and Kutscher [1931]. Their finding as to the incidence of creatine P is essentially in agreement with ours for C 180 tumour. They also confirm the statement of Barr, etc. [1928] that fluoride is without influence on the free P changes in the incubated tumour. Fluoride also inhibits glycolysis. This may otherwise increase the free P liberation similar to the absence of sugar, masking a characteristic fluoride effect of removing free P. Edlbacher and Kutscher's finding that tumour can liberate free P from nucleic acid probably accounts for at least part of the increase in the acid-soluble P found by us. They also found some hydrolysis of hexosediphosphate by minced tumour. It is interesting to note that in all cases where preparations of tumour have successfully attacked a hexosephosphate [Neuberg, Downes, Edlbacher], either the magnesium salt or a solution containing magnesium has been used. Magnesium is known to be an essential element in coferment of muscle and yeast.

SUMMARY.

1. There is a trace of labile (creatine phosphorus) present in C 180 mouse tumour.

2. The increase in free phosphate of the tumour extracts on incubation is greater in the absence of added glucose than when glucose is present in the incubating fluid, thus showing a certain reciprocal relation to glycolysis.

3. During incubation of the mouse tumour there is an increase in acid-soluble phosphate. This is derived from the non-lipoid phosphorus of the precipitate. Only in the absence of glucose is the free phosphate liberated large enough to account for all of the new acid-soluble phosphate. There is thus an accumulation of an acid-soluble organic phosphate during incubation of the tumour in the presence of glucose when glycolysis is large.

4. From fractional precipitation of barium salts by alcohol no evidence is obtained of a shift from a hexosemonophosphate to hexosediphosphate during glycolysis of surviving tumour. The organic phosphate which accumulates only in the presence of glucose is precipitable as a single fraction by 20 p.c. alcohol in presence of Ba.

5. The "enzyme hydrolysable" phosphate of minced mouse muscle consists of at least two separate fractions. There is some evidence that the more-soluble may be further differentiated.

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STUDIES ON THE PHYSIOLOGY OF REPRODUCTION.

II. The effect of thymectomy on the age of puberty in the male rat.

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INTRODUCTION.

THYMECTOMY in the young rat has been shown to have no effect on the ability of the rat to develop adult testes which appear normal on microscopic examination [Pappenheimer, 1914]. No study on the effect of thymectomy upon the age of puberty in the male has been found, however. The fact that the age involution of the thymus occurs at about the time of puberty suggests the possibility of some relationship.

The removal of the thymus at an early age has been shown in a preceding paper [Andersen, 1932] to have no effect on the age of puberty in the female. The male litter mates of the animals used in Series II of that experiment were considered good material for a similar study in the male. The rats were thymectomized at 21 days. The operation, diet and care were identical for both sexes and are described in the preceding paper. Forty rats were thymectomized and 41 were kept as controls.

TECHNIQUE.

There is no criterion for the age of puberty in the male rat which is as definite and as easily observed as the opening of the vagina in the female. A brief survey of the literature revealed no standard method, and it was necessary to devise one. The observation of mating behaviour in the living animal is tedious and involves a large number of females, and the expenditure of a great deal of time in observing mating animals. The other possible method, which was adopted, was to kill groups of animals at various ages and inspect the genital organs. It was felt that the presence of motile sperm in a hanging drop preparation of a smear from the tail of the epididymis suspended in normal salt solution offered

a criterion which was both accurate and technically possible. A preliminary study of this method revealed that in rats of 40–50 days there were often a few non-motile spermatozoa with large heads and short tails in the head of the epididymis, whereas none were found in the tail. At about 50 days a few of these immature sperm were found in both head and tail. The rats which were 50–60 days old sometimes had large numbers of spermatozoa of the mature form which were non-motile, or only a few of which were motile. Most of the rats over 65 days of age had great masses of mature motile spermatozoa in the epididymis. On cutting the tip of the tail of the epididymis of the immature rats, no fluid was found, but in the mature ones several drops of viscous white material exuded and it was possible to diagnose the presence of the spermatozoa in this way. In every case, however, the fresh smears were studied microscopically. The epididymis from both sides was examined in all the animals and occasionally spermatozoa were found in one and not in the other.

The animals were autopsied at various ages between 50 and 115 days at approximately 5-day intervals. In each case the litter-mate control was killed on the same day as the operated animal. The record included the age and body weight at death, the result of examination of the epididymal smears and the weight to the nearest mg. of several organs: namely, the testes (separated from the epididymis), the thyroid, adrenal, spleen and thymus (in the controls). The thymic area and the entire neck of the operated animals were examined for remaining pieces of thymus tissue, which were not found in any case. Microscopic sections were made of the testes of all the animals killed between the ages of 50 and 69 days.

The effect of season on the age of puberty which was noted in the females in a previous paper was not studied in the males. The animals of this series were born in April and May. The female rats which were born at this time reached puberty at an earlier age than those born in November and December. It is therefore probable that the present data represented an earlier age of puberty in the males than would have been found in the winter-born animals.

Data.

Great individual variation both in the age of puberty and in the organ weights was found. The youngest rats were those killed at 50 days. Of these two of five operated animals, and one of seven controls had

motile sperm. Of the next age group, 55 days, all of seven operated animals had a few non-motile sperm, while one of three controls had many motile sperm, and the others no sperm in the epididymis. Beginning at 60 days nearly all the animals had mature motile sperm. We may then estimate the usual age of puberty of rats of our stock breed, fed on our standard diet and born in the late spring, as about 60 days. This is about 2 weeks later than the arrival of puberty in their female litter mates, as judged by the opening of the vagina. There is no apparent difference in the age of puberty between the males which were thymectomized and their controls.

The absolute and relative weights of the thyroid, adrenals, spleen and testes vary within approximately the same range in corresponding age groups in the operated and control rats. The number of rats in each age group is too small for the data to be treated statistically. The mean relative weights of the organs of the operated and control animals of each age group are given in Table I. There is a fair degree of variation in

TABLE I. The mean relative weights of various organs at different ages in thymectomized and control male rats.

Age in days	Operated or Control	No. of rats	Body weight g.	G. per kg. body weight				
				Testes	Thyroid	Adrenal	Spleen	Thymus
50–54	Op.	5	122·8	15·2	0·094	0·222	3·20	
	C.	7	122·7	12·9	0·090	0·205	5·38	3·10
55–59	Op.	7	126·0	13·1	0·100	0·159	6·00	
	C.	3	124·7	12·4	0·129	0·150	9·22	3·54
60–64	Op.	5	144·8	15·7	0·083	0·158	5·06	
	C.	8	146·9	13·4	0·094	0·146	6·69	2·91
65–69	Op.	6	141·7	14·9	0·099	0·162	4·21	
	C.	7	158·0	14·3	0·098	0·147	4·99	2·28
70–74	Op.	7	149·9	13·1	0·103	0·152	5·31	
	C.	5	152·3	12·0	0·113	0·151	6·50	2·13
95–99	Op.	3	193·0	12·3	0·086	0·119	2·25	
	C.	3	198·9	11·4	0·083	0·112	2·62	1·61
100–104	Op.	3	208·3	10·5	0·072	0·123	3·71	
	C.	3	209·3	9·5	0·075	0·144	5·19	1·33
105–109	Op.	2	233·0	9·9	0·065	0·116	2·80	
	C.	3	241·0	9·9	0·064	0·126	3·15	1·43
115	Op.	2	259·0	10·2	0·073	0·082	3·02	
	C.	2	228·0	10·8	0·096	0·097	3·31	1·18

organ weights in each series, which was in part due to the error attendant on dissecting small organs and in part to normal variation. The weights were believed to be accurate to 1 mg. The relative weights of the testes, adrenals and thymus appear to decrease between the ages of 50 and 100 days. The relative weights of both testes are 11 to 12 g. per kg. of body

weight in rats in which only a few slightly motile, or non-motile sperm or no sperm were found in the smear. Where the smear contained masses of motile sperm, the testes weighed 13–15 g., usually 14 g. per kg.

In all of the rats which were killed before the age of 70 days the testes were studied histologically. In each case spermatozoa were found in the tubules. When the epididymal smear and the section of testis were compared it was found that in each rat in which the epididymis contained masses of mature motile sperm the testis was that of a mature animal: the tubules contained many cells with mitotic figures, and the lumina contained great numbers of free sperm. Where the epididymal smear contained either no sperm or a few non-motile sperm the testis was immature, with only occasional sperm in the lumina, a few young sperm with their heads still deep in the wall of the tubule, and many tubules without spermatozoa. No difference was observed between the testes of the operated and those of the control rats.

Discussion.

The method of ascertaining the maturity of the rat by the presence of a great number of mature motile sperm in the tail of the epididymis is satisfactory for accuracy and ease of execution. It requires, however, a large series of animals. The age at puberty cannot be determined as accurately for the male as for the female animals. It seems safe to conclude from this experiment that the age of puberty in the male is not affected by thymectomy at the age of 21 days.

Further discussion of the relation of the thymus to reproduction may be found in the previous report on thymectomy in the female rat and in the review of the literature, which are now being published [Andersen, 1932].

Summary.

A series of 40 male rats were thymectomized at the age of 21-22 days and killed at various ages between 50 and 115 days. The tail of the epididymis was examined by a hanging-drop preparation of the smear for the presence of mature motile spermatozoa. The thyroid, adrenals, spleen, testes and (in the controls) the thymus were weighed for each rat. This series was controlled by 41 male rats from the same litters and killed at the same ages.

Conclusions.

1. Thymectomy at 21 days does not alter the age of puberty in male rats as judged by the presence of many mature motile spermatozoa in the tail of the epididymis.

2. The age of puberty in the male albino rat under the conditions of this experiment is about 60 days.

3. The actual and relative weights of the thyroid, adrenals, spleen and testes in operated and control rats of the same age vary within the same range.

4. The testes of rats with many mature motile spermatozoa in the epididymis are heavier than those in the animals in which the epididymal smear shows a few non-motile sperm or no sperm.

5. The relative weights of the thymus and of the adrenals in both the control and the operated series decrease with increasing age between the ages of 50 and 115 days.

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STUDIES ON THE PHYSIOLOGY OF REPRODUCTION.

III. The effect of thymectomy on fertility in the rat.

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In the course of some experiments on the effect of thymectomy on the age at puberty which have been recently reported [Andersen, 1932], 54 of the rats were kept for mating. The diet and care of the animals are discussed in the previous papers. Four operated and 4 control rats of each sex were from Series I and were born in the winter; the remainder, consisting of 8 operated and 7 control males and 12 operated and 11 control females, were born during April and May. All the rats were thymectomized at the age of about 21 days and were first mated at the age of 3–5 months. Each rat was mated several times and at least one mating was with a rat known to be fertile. An interval of rest lasting 1–2 weeks was allowed between matings. After two or three matings the animal was autopsied and the mediastinum and neck were examined for gross fragments of thymus. None were found. The operative and autopsy technique are discussed in the preceding reports.

The data are recorded in Table I. Of the operated animals 75 p.c. of

TABLE I. The fertility of thymectomized rats.

		Operated		Controls	
		Fertile	Sterile	Fertile	Sterile
Males:	Series I	3	1	3	1
	Series II	6	2	5	2
	Totals	9	3	8	3
Females:	Series I	4	0	4	0
	Series II	8	4	10	1
	Totals	12	4	14	1

the males and 75 p.c. of the females were fertile. Of the controls, 73 p.c. of the males and 93 p.c. of the females were fertile. In view of the smallness of the series this difference is not significant.

The previous reports of pregnancy in thymectomized animals have

been few. The largest number of animals were reported by Paton [1905] who found that the first pregnancy occurred at about the same age in 8 operated and 6 control guinea-pigs. Other examples of pregnancy occurring in female animals following thymectomy are given by Fischl [1907] in 2 chicks; by Paton and Goodall [1904] in 2 guinea-pigs; and by Nordmann [1914] in 2 dogs. No study on fertility in the male after thymectomy has been found. In none of these reports was any mention made of examination of the thymic area for fragments of the gland.

In view of the previous negative findings in regard to the effect of thymectomy on the age of puberty, it is unlikely that the operation would affect fertility. The present observations contribute additional evidence against any specific thymic-gonadal relationship.

Conclusion.

Thymectomy has no effect on fertility in either the male or the female rat.

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THE RENAL CIRCULATION RATE IN THE RABBIT.

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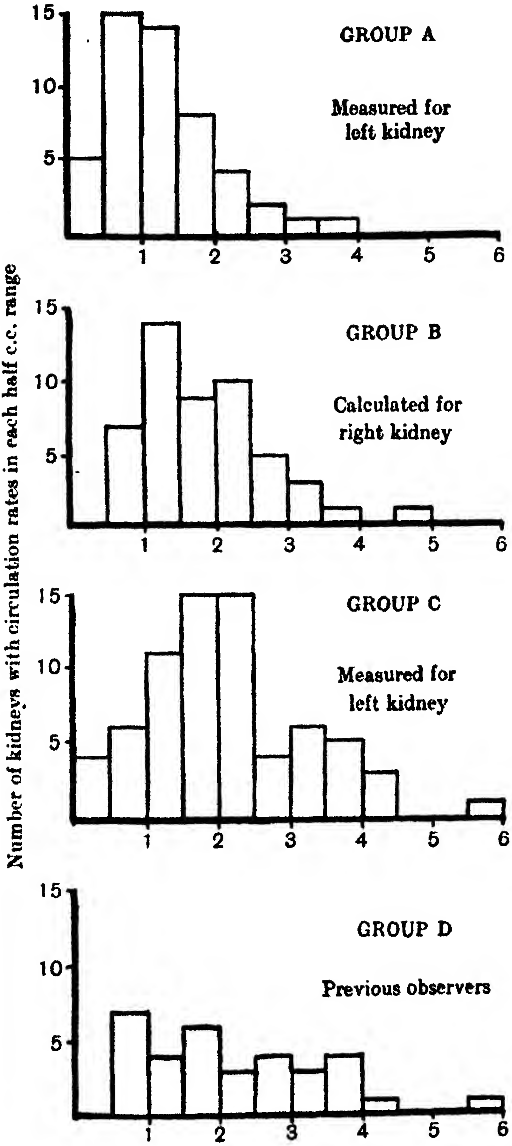
IN calculations about the function of the kidneys it is often necessary to make an assumption as to the probable amount of the renal blood flow. The figures suggested by different writers are somewhat varied, and in recent years there has been a tendency to assess the renal blood flow at a higher level than formerly. This is prompted to some extent by the results of perfusion experiments on the excised kidney, though it is recognized that the large blood flows in such experiments are not directly applicable to the intact animal. It is of interest therefore to review the figures from a fairly large series of measurements of the renal blood flow in rabbits which, as far as can be ascertained, appear to have been little disturbed by the short operative interference.

In group A are collected the circulation rates through the left kidney in a series of fifty experiments with dyes. Many of these experiments have been reported previously [Sheehan, 1931]: the method employed was described under the term "long sample experiment." The blood was collected directly from the renal vein by a hypodermic syringe for a measured time varying from 30 to 70 sec.; the volume of blood collected was from 4 to 22 c.c. In certain experiments the flow of blood became slower towards the end of the collection; the circulation rates for these kidneys have been calculated from the time of collection of the first 4 c.c. of blood. The mean circulation rate of the group is 1·4 c.c. per g. per min.

It was pointed out in that paper that the operative technique produced some vaso-constriction of the left kidney, but that the blood flow through the untouched kidney of the opposite side could be calculated from the relative amounts of dye in the two kidneys. The circulation rates are not directly proportional to the amounts of dye in the kidneys, since the renal extraction ratio for the dyes diminishes with increasing renal circulation rates. For accuracy therefore a factor has to be introduced to allow for the degree of inverse relationship between the renal



Renal circulation rate. c.c. per gm. per min.

Fig. 1.

extraction ratio of the particular dye used in the experiment and the renal circulation rate. The figures obtained by calculations on these lines for the circulation rates of the right kidneys of the fifty animals of group A are given in group B. The mean rate for this group is 1·9 c.c. per g. per min.

In group C are collected the figures obtained in a series of seventy experiments in which the venous blood flow from the left kidney was directly measured by an improvement of the previous method. The technique has been described recently in connection with the results of a few of the experiments [Dunn, Kay and Sheehan, 1931]; the quantity of blood collected is only from 4 to 5 c.c., the time required is shorter and the interference with the renal pedicle is much less than in group A. Some of the animals had artificially raised blood ureas, but the group has not been subdivided since no effect of urea on the renal circulation rate has been established. In ten of the experiments the blood flow became obviously slower towards the end of the collection; the rate has been calculated in these cases from the time of collection of the first 2 c.c. of blood. The mean renal circulation rate is 2·0 c.c. per g. per min.

Group D shows for comparison the combined figures of the following observers:

		Number of rabbits	Mean renal circulation rate c.c. per g. per min.
Barcroft and Straub	1910	4	1·8
Tribe and Barcroft	1916	7	2·0
Dunn, Dible, Jones and Mc Swiney	1925	10	1·4
Hayman and Starr	1925	8	2·6
Wagoner and Livingston	1928	1	5·8
Livingston	1928	3	3·5

Certain of these investigators employed methods which involved extensive evisceration and a consequent focussing of the circulation on to the kidney, but which avoided any touching of the renal pedicle. In addition it is probable that some of the workers give only a selection of the figures they have obtained. It is open to question whether their results are as representative of the normal as those obtained in the relatively intact animal. The mean renal circulation rate for group D is 2·2 c.c. per g. per min.

DISCUSSION.

Groups A, B and C are unselected and include all the low as well as the high blood flows that have been measured in normal animals. The only experiments that have been discarded are those in which the operation was not satisfactorily performed. The method used in groups A and C has the disadvantage of involving the insertion of a needle into the renal vein, but there is no interference with the arterial system as in tying large arteries or taking blood-pressure measurements. In group A the interference with the renal pedicle produced some reflex renal vaso-constriction rather frequently, and this group cannot therefore be considered to show the normal range. Groups B and C appear less open to this objection; they show a reasonably close agreement and may be taken as a basis for further discussion. The averages for the two groups combined are as follows:

Renal circulation rate c.c. per g. per min.	0–1	1–2	2–3	3–4	4–5	5–6
Percentage of animals	14	41	29	12	3	1

The mean of all the rates of the two groups is 2·0 c.c. per g. per min. The problem that arises immediately is whether these findings represent the conditions obtaining in the intact animal or whether they are merely an indication of varying degrees of renal vaso-constriction as a reflex from the needling of the vein.

Some general information as to the renal blood flow can be obtained from the appearance of the untouched kidney immediately on opening the animal. With a sufficient experience of the operation it is possible to recognize differences in degree between the circulation through the kidneys of different animals from the colour of the kidneys and from the size of the renal artery. The colour of the kidney depends on the amount of blood in the intertubular capillaries and on the degree of oxygenation of this blood. In most animals the kidney is of a certain "usual" redness, in a few it is pale and livid brown, while in a few others it is bright scarlet. Associated with these types of kidneys there are corresponding conditions of the renal artery, which in most animals is of "usual" size, occasionally is narrow and inconspicuous, and occasionally is widely dilated and bright red. No precise measure of the "usual" appearances is of course possible, but the two extremes are very striking and obvious. Observations of this kind on all the animals of group C make it clear that the circulation rates through the untouched kidneys vary within a wide range, but that a very large renal blood flow is not common. In

most animals the blood flow as measured by the subsequent aspiration is in agreement with the blood flow as roughly estimated from the appearance of the kidney. Occasionally, however, a definite retardation of the blood flow develops. This is indicated in two ways: the times for the collection of each successive c.c. of blood become progressively longer, and there is an obvious blanching of the kidney. The condition may be very well demonstrated by artificial interference with the supply of arterial blood to the kidney, *e.g.* by needling the heart during the collection of the renal vein blood.

Any serious vaso-constriction can thus be easily recognized, and, as mentioned previously, in the few cases in which it did occur the renal circulation rate has been calculated from the blood flow only in the early stages of the aspiration. It may therefore be accepted that the great variation of the rates in group C is not dependent on any commonly occurring gross reflex vaso-constriction from the aspiration. It is more difficult to assess the possibility of the occurrence of minor degrees of vaso-constriction. The comparison of the dye contents of the two kidneys in group A indicated that the collection of renal vein blood in that group produced some reflex diminution of blood flow to the left kidney. By analogy it might therefore be considered probable that a similar vaso-constriction occurred in group C; though of slighter degree, since the interference with the renal pedicle was much less. In addition the occasional occurrence of obvious reflex vaso-constriction in group C suggests that a reflex vaso-constriction, too small to be obvious, may have been rather commonly present. It seems, however, most unlikely that any vaso-constriction which was not large enough for recognition can have produced more than a 25 p.c. reduction of the renal blood flow. The comparison of groups B and C does not suggest that there was any vaso-constriction in the latter group. It may be concluded therefore that the renal circulation rates in the anæsthetized animal before operation were probably little if any higher than those measured. Further it has been shown, in the paper on dye deposition referred to previously, that urethane does not seem to influence the blood flow through the kidney. The renal circulation rates found in the present series may thus be taken to represent fairly closely the rates obtaining in the intact normal animal. The mean for the intact animal appears to be 2·0 c.c., or possibly up to 2·5 c.c. per g. per min.

Certain other considerations appear reasonable. It is difficult to accept that figures of 0·5 c.c. or 5·0 c.c. per g. per min., measured over a few seconds only, mean anything but temporary spontaneous fluctuations

of the renal blood flow. If seventy separate measurements of the renal circulation rate could be performed on one rabbit at different times, the range found would probably be similar to that shown in group C for one measurement on each of seventy rabbits. Further, the mean rate not only represents the average for a number of different rabbits but it probably also approximates to the average for each of these rabbits over a period of 24 hours.

The view that the renal circulation rate averaged over the whole day is about 2 c.c. per g. per min. in the intact rabbit finds some support from the investigations on the excretion of urea which have been referred to earlier [Dunn, Kay and Sheehan, 1931]. The following are the mean figures from eighteen rabbits on which satisfactory determinations were made. It was found experimentally that each 100 c.c. of blood, containing originally 30·2 mg. of urea, lost 2·5 mg. of urea in passing through the kidneys. During the previous few days the urinary excretion of urea was 0·56 mg. per min. This would require during the previous few days a mean renal blood flow of 22·4 c.c. per min. or, since the kidneys together weighed 11·5 g., a renal circulation rate of 1·95 c.c. per g. per min. The mean rate actually obtained in the experiments was 1·9 c.c. per g. per min.

These animals were excreting rather less urea than is usually the case under the conditions of this laboratory; the renal circulation rates needed to excrete this urea are thus perhaps a little lower than usual. As an example may be given the averages from a number of other rabbits, in each of which the daily urinary output of urea was measured over a period of 9 days. No renal vein operation was performed; the renal circulation rates are calculated on the assumption that the mean renal extraction ratio for urea was the same as in the previous animals.

Number of rabbits	Body weight kg.	Urinary urea output mg. per min.	Mean weight of kidneys g.	Calculated renal circulation rate c.c. per g. per min.
9	1·0–1·4	0·69	10·1	2·7
9	1·5–1·9	0·71	12·3	2·3
24	2·0–2·4	0·92	16·3	2·2
6	2·5–2·9	0·90	19·0	1·9

The mean rate for this series is 2·3 c.c. per g. per min. The occurrence of higher mean rates for the small animals than for the larger ones is not significant and depends on the small numbers of animals. No such relationship of body weight to renal circulation rate can be traced in groups B and C.

SUMMARY.

Measurements of the renal circulation rate have been made in 120 rabbits. The rate is variable; it is usually between 1 and 3 c.c. per g. per min., and is sometimes as high as 6 c.c. per g. per min., though this figure is exceptional. The mean rate is 2 c.c. per g. per min.

It appears that the mean rate for the intact animal over a period of 24 hours is from 2 to perhaps 2·5 c.c. per g. per min.

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612.745.3

THE DELAYED ANAEROBIC HEAT PRODUCTION OF STIMULATED MUSCLE.

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THE production of a considerable quantity of heat following a short tetanic contraction under strictly anaerobic conditions has been constantly observed, and during the past ten years has been the subject of a number of investigations from these laboratories [Hartree and Hill, 1922, 1923; Furusawa and Hartree, 1926; Hartree and Hill, 1928; Hartree, 1929; Blaschko, 1930]. While variable in magnitude the delayed anaerobic heat has been an easily demonstrable phenomenon which could not be explained by some peculiarity of the technique. It appeared to represent a true physiological activity on the part of the muscle, although its significance remained questionable. With the confirmation of the findings of Embden and his associates [1926, 1927] through the recent investigations of Lehnartz [1931], of Lundsgaard [1931], and of Meyerhof and Schulz [1931], showing that in a short tetanus a large fraction of the total lactic acid production occurs after the contraction is over, the delayed anaerobic heat production receives a definite place in the chain of events associated with muscular activity. It becomes, therefore, of special interest to inquire further into the conditions which influence the magnitude and time course of the delayed anaerobic heat, which recent refinements in technique have made possible to an accuracy hitherto unobtainable. The present study has been taken up from this point of view with particular reference to the delayed heat following a series of twitches, and the changes occurring with fatigue.

THE DELAYED ANAEROBIC HEAT FOLLOWING A SERIES OF TWITCHES.

For the determination of the delayed anaerobic heat production resulting from a series of twitches the same general technique employed in this laboratory during the past few years has been utilized [see Hill,

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1928*a*]. Single or double sartorius preparations from English *R. temp.* were mounted on the thermopile (silver-constantan couples, shellac-bakelite insulation and a glass cover) and connected with an isometric lever recording on a smoked drum. The muscle chamber was placed in a thermostat at 20·3° C., constant to 0·001°, where it was allowed to remain with the muscle soaking in oxygenated Ringer's fluid (*p*H 7·2, phosphate 10 mg. P/100 c.c.) for about 2 hours. This preliminary soaking in Ringer's fluid insured the retention of the normal irritability of the muscle, and at the same time permitted the equalization of temperature throughout the chamber. At the end of this period the oxygen was replaced by nitrogen (purified by passing over heated copper) and the Ringer's fluid by a good grade of oxygen-free paraffin oil, previously brought to the same temperature as the rest of the system. A further period of from 30 to 50 min. was allowed to insure complete freedom from oxygen and a stable galvanometer zero. During the actual observations the slow passage of nitrogen through the oil was discontinued, as it was found that by so doing the accuracy of the readings was improved.

As soon as a steady base line was obtained a series of maximal single shocks was given at the desired frequency, and the total heat determined by the area of the galvanometer deflection-time curve [see Hill, 1928*a*, p. 120]. At the end of the experiment the muscle was killed by a short period of overstimulation, and when stable temperature conditions had again been reached the time-deflection curve to control heating of the same duration was obtained. On the assumption that no considerable amount of delayed heat is produced during the period of contraction[1] the difference between the values of the area of the live and the control curves (after correcting to the same maximum) gives the total delayed heat. Usually, however, we have employed the alternative procedure of analyzing the live curves by means of the controls according to the method described by Hill [1929] in connection with a study of the heat production of crustacean nerve, and by Bronk [1930] for the analysis of the heat liberated by a muscle during a sustained contraction. For such an analysis the period of control heating was made the same as the time units in which the analysis was to be carried out.

With the procedure described above it has been possible to record small temperature changes over long periods to an accuracy greater than previously attained. By completely immersing the muscle in paraffin oil all temperature changes due to water condensation have been pre-

[1] *I.e.* delayed heat attributable to the earlier contractions of the series.

vented, and thus the necessity for the rather large correction due to changes in vapour pressure has been eliminated [see Hill and Kupalov, 1930]. Moreover the high viscosity, low specific heat, and low conductivity of the oil all contribute to protect the thermopile from adventitious changes in temperature. The result has been an extremely constant galvanometer zero continuing over long periods of time. The galvanometer was critically damped and adjusted in each experiment to a sensitivity giving a deflection of between 200 and 600 mm. on the scale placed 3 metres from the mirror. Under these conditions the beam of light, which was read to $\frac{1}{4}$ mm., could as a rule be counted upon to return to within $\frac{1}{2}$ mm. of its initial position following a series of twitches. With this constancy it has been possible to measure accurately the very small rate of heat production occurring for from 10 to 20 min. following stimulation, and this has resulted in the demonstration of a definite amount of delayed heat associated with a series of single twitches which in the earlier work of Hill [1928*b*] escaped detection.

The results are summarized in Table I, which includes the data from every experiment performed on the delayed heat in a series of twitches. It will be seen that following a series of single stimuli numbering from 20 to 240 there occurs quite regularly the formation of extra heat amounting to from 10 to 20 p.c. of the value of the initial heat. This is of the same order of magnitude as the values previously reported for the delayed anaerobic heat production following a short tetanic stimulus. In Exps. 18 and 19 the delayed heat for a series of twitches and a short tetanus have been determined in the same muscle, and here also there is a rough agreement in values. In general the delayed heat as a percentage of the initial heat, or of the total energy liberated, is less for the longer series of twitches, although its absolute value is greater. In certain experiments, *e.g.* Nos. 13 and 14, in which several observations have been made in the same muscle, this relationship is clearly shown. If, however, the amount of activity is still further reduced so as to involve less than about 20 twitches the amount of delayed heat, both absolutely and as percentage, becomes less and drops to a very low value. A similar change has been observed in a few experiments with tetanic stimulation, described in the next section of this paper: with a duration of 0·1 sec. or less, the amount of delayed heat is insignificant. A further point shown by the data in Table I, additional evidence for which will be given later, is that when the muscle becomes fatigued, as indicated by the falling off in tension and total heat production, the percentage value of the delayed heat becomes less.

TABLE I. Summary of experiments showing the amount of delayed anaerobic heat as a percentage of the initial heat. The maximum galvanometer deflection shows the relative amount of energy (initial heat) liberated in successive observations on the same muscle, but does not give a comparison for different experiments since the galvanometer sensitivity, etc. was not the same.

Exp.	Stimulation: Rate: shocks per min.	Stimulation: Duration	Maximum deflection	Duration of delayed heat: min.	Delayed heat as p.c. of initial	Remarks
9	60	4 min.	370	18	7·3	2 min. rest after second min.
10	60	4 ,,	443	—	—	
	60	4 ,,	281	12	3·5	Tension poorly maintained
11	60	2 ,,	260	17	10·3	
	60	2 ,,	173	19	11·0	
13	56	0·4 min.	304	12	21·5	
	56	0·8 ,,	488	13	11·9	
	56	1·6 ,,	603	19	9·4	
	56	0·8 ,,	298	9	5·6	Marked reduction in tension
14	63	0·4 ,,	480	12	21·5	
	63	0·4 ,,	488	12	22·2	
	63	0·8 ,,	627	13	12·3	
	63	1·2 ,,	454	10	7·4	Marked reduction in tension
15	100	0·4 ,,	523	8 (?)	8·5 (?)	Poor galvanometer zero
	100	0·8 ,,	835	17	9·5	
	100	0·4 ,,	537	8	7·8	
16	100	0·2 ,,	258	9	16·5	
	100	0·4 ,,	450	10	19·5	
	100	0·2 ,,	—	8	16·3	
17	100	0·2 ,,	342	7	13·5	
	100	0·4 ,,	585	10	10·6	
	100	0·2 ,,	479	8	10·4	
18	105	0·2 ,,	374	8	11·2	
	Tetanus	1 sec.	356	8	11·6	
	110	0·2 min.	414	8	10·9	
	Tetanus	1·6 sec.	272	6	7·8	
19	56	0·4 min.	296	8	12·9	
	Tetanus	1 sec.	719	15	22·8	
	56	0·4 min.	213	9	12·5	
	Tetanus	1 sec.	637	18	18·0	
20	55	0·2 min.	230	4	3·3	
	43	0·4 ,,	179	7	13·0	
	35	0·2 ,,	152	6	8·9	
21	60	0·2 ,,	318	7	6·0	
	60	0·2 ,,	336	6	1·6	
	60	0·2 ,,	340	6	4·5	
	60	0·4 ,,	561	8	13·0	

Through the method of curve analysis the time course of the delayed heat production may be determined with considerable accuracy, and this has been done for all the experiments. A very small and constantly decreasing rate of heat production continues over periods ranging between 5 and 20 min. after the contractions are over. The total duration of the

delayed heat production is shown for each experiment in Table I; in general it is directly related to the amount of the previous activity, the longest times being observed for the longer series of twitches and in the tetanic contractions involving the larger energy expenditure. When the actual heat production for each unit of time is determined by analysis the delayed heat production is found to have already reached its maximum rate immediately on cessation of activity, at the end of the series of twitches lasting for 24 sec. or longer, and to fall continuously thereafter

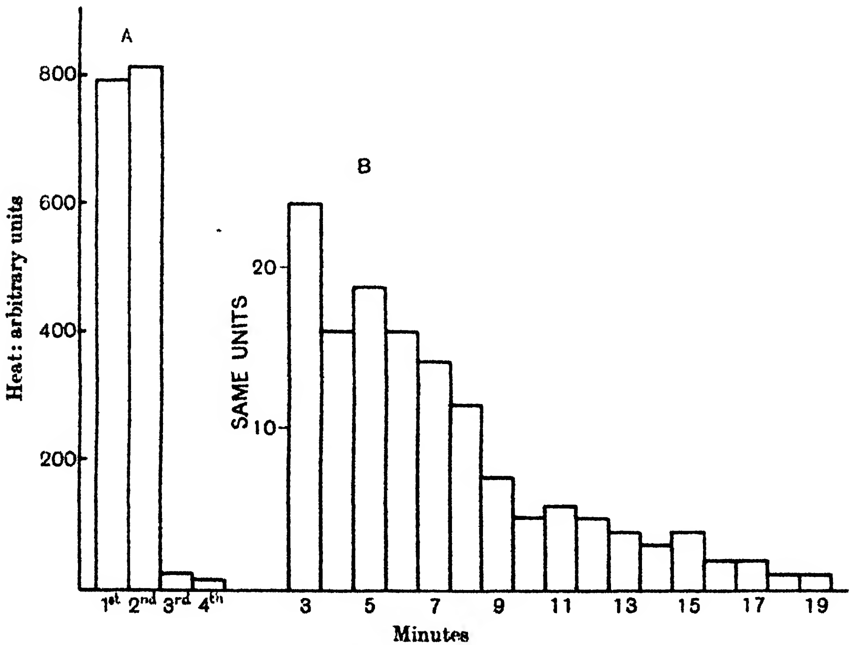

Fig. 1. Analysis of galvanometer deflection in Exp. 11. 120 single shocks in 2 min. Each block represents the heat given out during the corresponding minute. Analysis as made with no remainders. A, showing initial heat and first two blocks of delayed heat for comparison of the rate. B, continuation of the analysis, drawn on an enlarged scale, showing distribution of the delayed heat production from the end of the stimulation period until it ceased 17 min. later.

in the general form of a logarithmic curve. The results of such an analysis are charted in Fig. 1. In the case of a tetanus lasting only a second or less the delayed heat production is still at a very low value immediately following activity, and does not reach its maximum rate until from 20 to 40 sec. later; in the present experiments, however, with a series of twitches, where we have no means of separating the delayed from the

initial heat during the period of activity, the duration of activity was of such length that it obscured the rising phase of the rate of delayed heat production. A correction has been made for the small quantity thus lost by assuming a regular increase from zero to the value found for the time unit immediately at the end of the series of twitches, the calculated value for which was added to the delayed heat and subtracted from the initial heat.

THE ANAEROBIC DELAYED HEAT PRODUCTION AFTER A SHORT TETANUS.

The experiments under this heading were carried out at Cambridge at an earlier date than those just described. The same general technique was employed, except that the muscle was mounted on a constantan-iron thermopile (shellac insulation, vulcanite chamber), and after a preliminary period of soaking in Ringer's fluid, the determinations were made with the muscle in nitrogen, without the use of oil. Under these conditions there is always a small permanent positive deflection of the galvanometer following stimulation of the muscle, due to increased osmotic pressure [Hill and Kupalov, 1930]. In order to obtain the true value of the delayed anaerobic heat it was necessary to make a correction for the temperature change from this source, which was done by subtracting the value of the permanent deflection from each point of the time-deflection curve. To obtain the initial values it was assumed that the entire increase in osmotic pressure occurred during the period of activity, when the form of its rise to maximum could be determined by the deflection resulting from a period of control heating, continued until a steady maximum deflection was reached.

The results from six experiments are given in Table II. In the fresh muscle, for a given amount of energy expended, the values for the delayed heat are of the same order as those already given for a series of twitches. (The energy expended in a series of 20 twitches at 20° C. is equal to that in a tetanus having a duration of from 1 to 2 sec.) In each experiment, in the later observations taken after a considerable amount of previous stimulation, from which recovery could not take place since the muscle was kept in nitrogen, the delayed heat was a much smaller percentage of the initial heat. In the six experiments of Table II, the average value of the delayed heat in the later contractions, expressed as a percentage of the initial heat (which also diminishes), is one-half the value for the fresh muscle. The actual course of this change with activity

TABLE II. The effect of activity on the delayed anaerobic heat, expressed as a percentage of the initial heat. The figures for the maximum deflection in each experiment show the decline in energy expenditure occurring between the first and the last series of trials. In the last two experiments the muscle was first poisoned with curare. All at 16½ to 17° C.

Exp.	Stimulation number	Duration of stimulus sec.	Maximum deflection	Delayed heat p.c.
7. i. 31	3rd and 4th	1	742	16
	7th and 8th	1	630	11
9. i. 31	1st and 2nd	1	1050	21·6
	23rd and 24th	1	510	8·2
13. i. 31	3rd and 4th	1	750	21·4
	11th and 12th	1	645	9·8
15. i. 31	2nd and 3rd	2	165	12·8
	12th and 15th	2	73	7·5
17. i. 31	7th and 9th	1	170	12
	12th and 13th	1	143	10
21. i. 31	4th and 5th	0·5	137	18
	14th and 15th	0·5	127	4

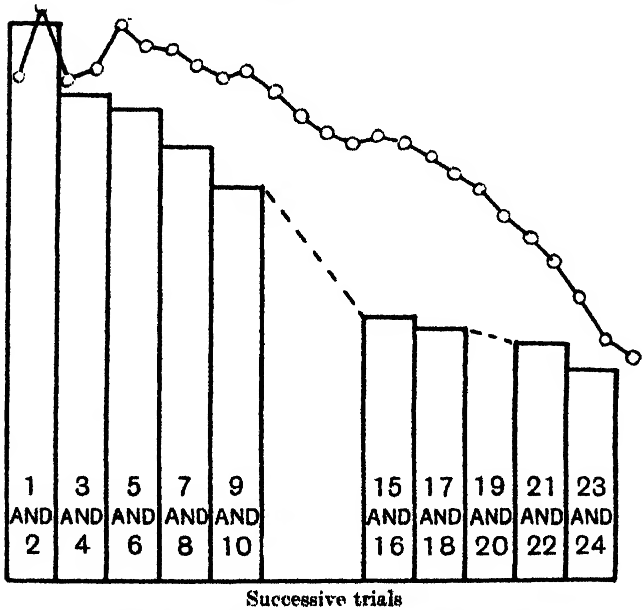

Fig. 2. Each block represents the total delayed heat resulting from a 1 sec. tetanic stimulus, expressed as a percentage of the initial heat, and is the average of two successive trials. The first block has a value of 21·6 p.c. and the last 8·2 p.c. The curve above gives the maximum galvanometer deflection, and shows the change in the initial heat occurring during the series.

is well shown in the example plotted in Fig. 2, in which the delayed heat was determined for 24 successive tetanic stimuli of 1 sec. each.

It is clear that with repeated stimulation the delayed anaerobic heat becomes less, but on the other hand, the changes do not necessarily run parallel with the decrease in total energy. It may be pointed out, for example, that in the experiment shown in Fig. 2 there was very little change in the total heat produced by the first 10 tetani, whereas the delayed heat decreased by about 25 p.c.; while later when the total heat was falling most rapidly, the value for the percentage of delayed heat remained nearly constant. Also in the last experiment of Table II there was a striking fall in the value of the delayed heat occurring in the course of the successive tetani, but the falling off in total energy was very slight. This suggests that the change in magnitude of the delayed heat is associated with some chemical change, which in its initial stages at least does not interfere with the power of developing tension.

In Table III are given the results of two experiments in which the

TABLE III.

Exp. of 19. i. 31. In a series of five trials to a 0·05 sec. tetanic stimulus the delayed heat averaged about 3 p.c. The duration of the stimulus was changed to 0·5 sec., and after an interval of about 1 hour a further series of determinations was made. This preparation showed very little fatigue, and the average value of the delayed heat for the 12th and 13th stimulations was 30 p.c.

Exp. of 21. i. 31. The delayed heat was first determined for a 0·5 sec. tetanus, and in four trials was about 18 p.c. of the initial heat (maximum deflection 1357 mm.). The duration of the stimulus was reduced to 0·1 sec. and a second series of five trials gave a mean value for the delayed heat of only 3·8 p.c. The delayed heat for a further series of stimuli of the original duration (0·5 sec.) was now determined, the first of which gave a value of 17 p.c. (maximum deflection 1360 mm.): the delayed heat became successively less, falling to a mean value of 4 p.c. (maximum deflection 1280 mm.) for the 4th and 5th trials. Note that the values for the maximum galvanometer reading were well maintained.

delayed heats (*a*) for a very short tetanus, and (*b*) for one of longer duration, were determined in the same muscle. In these experiments the delayed heat became but a very small percentage of the initial heat when the duration of the stimulus was reduced to 0·1 or 0·05 sec. This accords with the results obtained in a series of twitches (see Table I, Exps. 20 and 21) when the total energy expenditure was small.

DISCUSSION.

In the present state of our knowledge it is not possible to correlate with any exactness the time relations of energy liberation and mechanical change with the chemical events responsible for them. During the past few years, however, our conceptions have undergone a complete change, and considerable progress has been made towards an understanding of the energy relations involved in muscular contraction. For a recent

statement of the problem see Hill's Pennsylvania lectures [1931][1]. In the present connection we are particularly concerned with the production of lactic acid after the completion of the mechanical response, for in this process the delayed anaerobic heat production receives an adequate explanation. In various investigations from Embden's laboratory [see Embden, Hirsch-Kauffmann, Lehnartz and Deuticke, 1926; Embden, Lehnartz and Hentschel, 1927] it was shown that a considerable fraction of the total lactic acid formation, both in the case of a series of twitches and in a tetanic contraction, occurs after the cessation of activity. This observation, in so far as it concerns the lactic acid formation following a tetanus, has recently been confirmed by Meyerhof [1931] and by Lundsgaard [1931]. The magnitude of the delayed lactic acid formation is quite large, amounting in Lundsgaard's experiments with a 5 sec. tetanus to approximately 50 p.c. of the total, a value which would represent an amount of delayed heat considerably greater than that observed. It has, however, been demonstrated that, even in the absence of oxygen, restitution of phosphagen continues after the contraction is over [see Meyerhof and Lohman, 1927; Nachmansohn, 1928; Lundsgaard, 1931], and since this is an endothermic reaction, there is little reason to doubt that the delayed anaerobic heat production represents the balance between lactic acid formation and phosphagen resynthesis [see Lundsgaard, 1931].

Both Meyerhof and Lundsgaard have failed to confirm the existence of a delayed lactic acid formation following a series of twitches, although Lehnartz's experiments [1931] appear to show it: in view, however, of the considerable quantity of delayed heat liberated it seems fairly certain that lactic acid is formed. The results of the analysis of the galvanometer curves show that the actual rate of heat production is extremely small, the large percentage ultimately recorded being the result of its long continuance. The lactic acid determinations were made on muscles immediately after contraction and from 1 to 4 min. later, a period which, judging from the heat production, is insufficient to allow more than a relatively small change in the total lactic acid content to occur, a difference which is probably very close to the experimental error of the method. There is a second possibility that should be considered in this connection: it is known that phosphagen resynthesis takes place with great rapidity, and it may be that in a series of twitches this process keeps pace with the activity, so that it largely ceases with its discon-

[1] See also Hill's British Association Address: "The Revolution in Muscle Physiology." *Physiol. Reviews*. In press.

tinuance. Under these conditions (the absence of phosphagen resynthesis) a given amount of heat would represent a much smaller lactic acid formation, an amount which might be missed in chemical analysis.

SUMMARY.

1. A series of twitches in nitrogen is followed by a definite production of delayed heat of the same order as that previously observed in connection with a tetanus.

2. The maximum value of the delayed anaerobic heat is about 20 p.c. of the initial heat, and occurs with moderate activity (about 25 twitches). It becomes less when the activity is more prolonged, and drops to a very low value when only a few twitches are involved. The value of the delayed anaerobic heat in a tetanus also becomes very small when the duration of the stimulus is sufficiently reduced.

3. The duration of the delayed anaerobic heat production is directly related to the severity of activity, lasting up to 20 min. following 120 twitches and dropping to about 6 min. for 12 twitches.

4. The value of the delayed anaerobic heat, as a percentage of the initial heat, becomes progressively less with repeated activity in the absence of oxygen.

It is our pleasant duty to acknowledge our indebtedness to Prof. A. V. Hill, who suggested the problem and gave us much helpful advice throughout the course of the investigation.

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THE CONTROL OF CIRCULATION THROUGH THE LIVER.

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I. Introductory and historical.

The experimental results obtained, in attempts to investigate the circulation through the liver and its control by nervous and other influences, present unusual difficulties of interpretation, on account of the complex of factors involved. This can be seen by a study of the numerous experimental investigations, scattered over a period extending from the middle of last century to the present day.

We have, in the first place, the complication due to the double blood supply from the hepatic artery and the portal vein. These are not independent, though there are very different accounts of the nature and the extent of their communication. The latest and most convincing account, by Olds and Stafford [1930], shows that the majority of the branches of the hepatic artery pour their blood into the lobular capillary spaces or sinusoids, either by fine arterioles opening directly into this lobular plexus, or through communications therewith of the plexus of finer capillaries round the bile ducts. The walls of the larger blood vessels—arteries, portal and hepatic veins—are further supplied by nutrient arterial twigs. The communication between the arterial and portal systems is apparently limited to the plexus of sinusoids in the lobule, but is there very free, and sufficient to introduce an important complication into the interpretation of records of flow and pressure, in the portal vein especially. A second and greater complication is due to the fact that the pressure and flow in the portal vein are dependent on the rate at which blood enters it through the veins draining the stomach, intestine and spleen, and therefore on vaso-motor and other changes in those organs, as well as on variations in the tone of the portal branches. Lastly, changes in the pressure in the vena cava, with variations in the return of blood from the

periphery or in the efficiency of the heart, may be transmitted back through the liver and affect the pressure and rate of flow in the portal vein.

It is obvious that, under such conditions, the lateral pressure in the portal vein in its natural relations provides no index of the flow through that vessel. If a rise of portal pressure is caused by relaxation of the arteries supplying the stomach and intestines, it is accompanied by an increased flow; if it is due to constriction of the portal branches, or to back pressure from the vena cava, it is accompanied by a retarded flow. Similarly, a rise in the lateral pressure in the hepatic artery may be accompanied by a decreased or increased arterial flow to the liver, according as increased tone of the hepatic arterioles or rise in the general arterial pressure predominates in its production. Under such complicated conditions, it is hardly surprising that the effects of stimulating the splanchnic nerves or the hepatic plexus, or of injecting adrenaline into the general circulation, on the pressure and rate of flow of the blood in the portal vein of the living animal, should differ in detail in the observations recorded by different observers.

For these detailed differences the original papers must be consulted, of which a fairly complete list is given at the end of this paper. To illustrate the type of difficulty encountered, we may mention that Burton-Opitz [1910–13], who published an extensive series of investigations in which lateral pressures and rates of flow were recorded in the hepatic artery and the portal vein of the living dog, was for long unable to confirm the conclusions of earlier investigators [v. Basch, 1875; Bayliss and Starling, 1894; François-Franck and Hallion, 1896] as to an active constriction of the branches of the portal vein in response to stimulation of the splanchnic nerves or the hepatic plexus. He observed a rise of portal pressure, indeed, but found that this was accompanied by an increased rate of flow, and attributed it to the rise of general arterial pressure. Later, when he had confirmed the observation of Schmid [1909], that injection of adrenaline into the portal vein causes rise of portal pressure, he made further experiments with artificial perfusion of the portal vein under constant pressure, and thus verified the constrictor effect, on the portal branches, of stimulating the sympathetic nerve supply. The fact that stimulation of the sympathetic nerve supply, or appropriate injection of adrenaline, causes constriction of the branches of both portal vein and hepatic artery has been amply confirmed by the work of several subsequent observers [Edmunds, 1915, 1921, 1924; Clark, 1928; Griffith and Emery, 1930].

The point which chiefly concerns us, however, is the control, not of the inflow to the liver, but of the outflow of blood from it through the hepatic veins. In 1928 three of us (B., D. and R.) had spent some time in devising, for another purpose, a scheme of complete artificial perfusions of the dog's liver, in which the arterial perfusion was carried out by a pump, the portal vein was fed from a constant-level reservoir, the outflow from the vena cava was continuously recorded, and the whole liver was enclosed in a plethysmograph. It came early to our notice that small doses of adrenaline, whether injected into the portal or the hepatic arterial stream, even when constriction of the hepatic and portal branches caused a restriction of the inflow, habitually accelerated the outflow from the vena cava. Reviewing the earlier investigations in the light of this observation, we found several indications of this phenomenon, which the authors had interpreted as due to some active, contractile effect. Thus, Ikalowicz and Pal [1887, 1888] described an accelerated flow from the hepatic veins of the dog when the splanchnic nerves were stimulated; and the conditions of their later experiments seemed to prove that it was due to some vascular change in the liver itself, since small quantities of blood flowed, at each stimulation of the splanchnic nerves, from a liver with the arterial and portal inflows obstructed, until the organ was nearly emptied of blood. François-Franck and Hallion [1896] found that stimulation of the splanchnic nerves in the dog caused, in addition to rise of hepatic and portal pressures and shrinkage of the liver, a rise of pressure in the vena cava at the level of entry of the hepatic veins. They interpreted this as due to a squeezing out of blood by contraction of the hepatic veins. Macleod and Pearce [1914], under conditions which practically limited the flow into the cava, below the diaphragm, to the output from the liver, and enabled it to be measured, found this output accelerated when the hepatic plexus was stimulated. They concluded that the arterial pressure normally produced a turgescence in Glisson's capsule, which restricted the flow in the portal branches, and that the reduction of this tension, by arterial constriction, so accelerated the portal inflow that the total flow through the liver increased.

A series of investigations from the Vienna school [Mautner and Pick, 1915, 1922, 1929; Lampe and Méhes, 1926; Baer and Roessler, 1926], in which the liver was artificially perfused from the portal vein with saline solutions or diluted blood, brought to notice a mechanism (Sperre) controlling the outflow of blood from the hepatic veins, on which histamine and other "shock poisons" have a powerful constrictor action. The swelling of the dog's liver in peptone "shock" had already been described

by Thompson [1899]. The "Sperre" mechanism was described as present in the livers of the dog and cat, but not in those of rodents, and was mistakenly held responsible for the whole of the depressor effect of histamine in the carnivora. More recently the earlier statements, as to its presence in the cat, and as to its exclusive rôle in the histamine depressor action, have been corrected [Mautner and Pick, 1929]. Experiments by Baer and Roessler [1926], in which the dog's liver was perfused alternately from the portal vein and, in the reverse direction, from the vena cava, showed that adrenaline as well as histamine, in the concentrations in which both were used, produced contraction of the hepatic veins; normally, however, the predominant action of adrenaline was constriction of the portal, of histamine constriction of the hepatic branches. The latter effect was located by this group of observers in the smaller radicles of the hepatic veins.

Similar results were obtained by Simonds and Brandes [1929] by adding Witte's peptone to the fluid perfusing the liver from the portal vein, on the one hand, or the vena cava on the other. Experiments by McLaughlin [1928], in which the livers of dogs, cats, rabbits and guinea-pigs were perfused from the portal vein with Ringer's solution, often at room temperature and in some cases with livers kept for a day in the ice chest, showed only the constrictor effect of adrenaline on portal branches. An initial increase of outflow, seen occasionally in rabbits' livers when change was made to solution containing adrenaline, was attributed, probably correctly, to a mechanical factor.

The only definite suggestion which we had found, of a relaxation of the mechanism controlling the outflow from the dog's liver in response to adrenaline, was in two papers by Mautner [1924*a*, 1924*b*]. According to this observer, stimulation of the vagus nerve or injection of histamine prevented the swelling of the liver from back pressure when the pulmonary artery was obstructed, in the dog or the cat but not in the rabbit. Adrenaline, in dog or cat, removed the obstruction, so that the liver volume again responded normally to back pressure. We may note that the alleged presence of such a histamine-sensitive mechanism in the cat does not correspond to anything in our own experience, or in the more recent statement of Mautner himself with Pick [1929].

Our own experiments, made under conditions which eliminated all extraneous influences on the rates of arterial and portal inflow, soon gave us clear evidence that the mechanism restricting the outflow from the hepatic veins in the dog, and thrown into intense activity by histamine, is regularly and promptly relaxed by small doses of adrenaline, or by

stimulation of the sympathetic nerve supply. At this point, short of completion, the investigation was interrupted by the return of two of us (B. and R.) to America. Mention was made of the results in a lecture [Dale, 1929], but the investigation made no further progress till the present year (1931), in which two of us (D. and P.) found opportunity to resume it, and bring it to the position now presented. In the interval, however, two important papers have been published by Grab, Janssen and Rein [1929 *a*, *b*], who have used Rein's thermographic method to obtain simultaneous records of the rates of flow in the hepatic artery, in the portal vein, and in the vena cava above and below the liver, in dogs under chloralose. They were able thus to demonstrate that the rate of outflow from the liver is not always identical with the total inflow; it may be greater or less, the floating balance of blood in the liver being accordingly diminished or increased. Atropine caused a lasting increase of outflow. Small doses of adrenaline regularly caused the outflow to exceed the total inflow, so that as much as 59 p.c. of the weight of the bloodless liver might leave the organ during such a period of excess outflow. The occurrence of this effect having thus been independently demonstrated, under less artificial conditions, we resumed the study of its mechanism with added interest, and investigated further the question of its occurrence in other species than the dog.

II. Methods.

The methods which we have used for perfusing the liver and for obtaining quantitative records of changes in different parts of the system have undergone many modifications of detail during the long and interrupted course of the work. It will suffice to describe it in the form most recently used, with which most of the records chosen for illustration were obtained. Fig. 1 shows the general arrangement of the apparatus.

Perfusion through the hepatic artery was carried out by a pump of the type described by Dale and Schuster [1928]. The pump was, as usual, immersed in a thermostat bath, *B*, and surrounded by a spiral glass tube communicating with the inlet valve. At each suction stroke a volume of blood, determined by the throw of the pump lever, was drawn from the container receiving the blood after oxygenation, through the glass spiral, in which it acquired the temperature of the thermostat, into the pump. At the return stroke this volume was ejected through the output valve into a straight glass tube which, passing through the wall of the thermostat by a water-tight gland, was connected by a short length of thick-walled rubber tubing to the arterial cannula. A glass T-piece, inserted in the course of this rubber connection, had its free limb connected by pressure tubing, filled with Ringer's solution, to a mercury manometer, by which the lateral arterial pressure was recorded.

Perfusion through the portal vein was made from a reservoir, *V*, the height of which could be fixed at an appropriate level above the liver. This reservoir was continuously filled by an accessory pump, drawing another portion of blood from the container, *A*, receiving it

after oxygenation. The action of this pump was adjusted to deliver blood into the reservoir more quickly than it entered the liver through the portal vein, the excess being returned to the oxygenator by an overflow tube, which kept the level of blood in the reservoir constant. From the bottom of the reservoir blood for the portal perfusion was led to a glass spiral, immersed in the same thermostat as the arterial pump, by a rubber tube long enough to allow the height of the reservoir to be adjusted through a good range. The other end of the

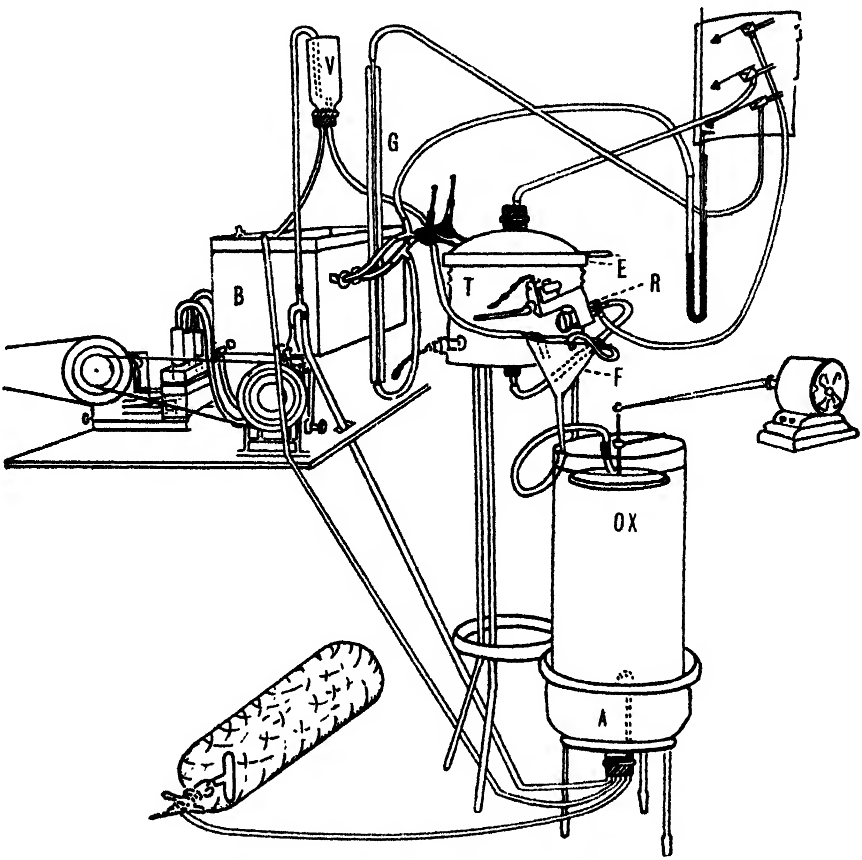

Fig. 1. General diagram of perfusion scheme.

glass spiral was connected to a straight glass tube traversing the wall of the thermostat, and this by a thick-walled rubber tube to the portal cannula. This rubber connection was interrupted by a glass T-piece, connected to a narrow, vertical glass tube, *G*, attached to a millimetre scale. In this manometer tube the blood rises to a height determined by the height of the blood surface in the reservoir, and by the rate of outflow from the portal cannula. If the outflow through the cannula is stopped, the blood column in the monometer rises, of course, to the height of that in the reservoir; the extent of its fall below this level gives an index of the rate of flow in the portal vein; and this, since the level in the reservoir is constant, is determined by the resistance in the flow system, and chiefly by that in the

liver. By applying artificial resistances to the outflow from the cannula, the manometer readings corresponding to different rates of flow could be determined at the end of an experiment and plotted on a graph, from which the change in flow corresponding to any change in manometer reading could be read. The top of the manometer tube was, further,

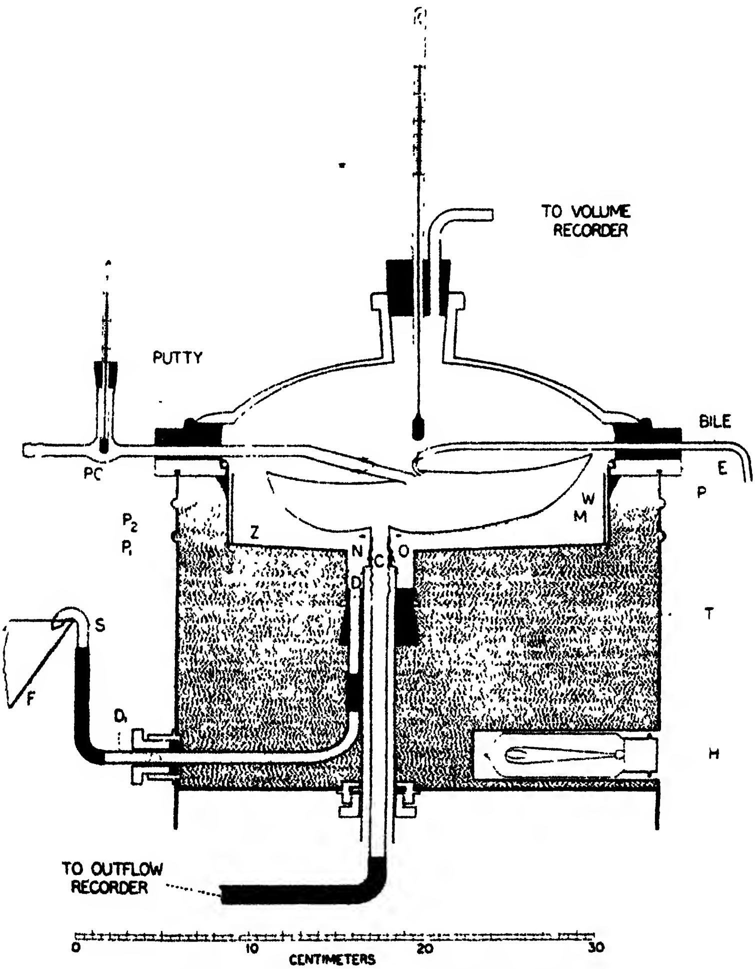

Fig. 2. Section of thermostat plethysmograph.

connected by a long rubber tube to a light bellows recorder writing on the drum, so that changes of portal flow were graphically recorded with those of arterial pressure.

The liver was enclosed in a vessel which served both as thermostat and air plethysmograph (Figs. 1 and 2). The large cylindrical copper tank, *T*, was filled with water kept at 40–41° by carbon-filament lamps introduced into the closed tunnel, *H*. A wide rim of thick ebonite, *E*, had on its under surface a deep circular groove, by which it fitted over the edge of the copper tank. The inner face of the ebonite rim was recessed, so as to give support to

the edge, strengthened with a stout wire, of the deep, circular zinc tray, Z, the junction between the tray and the ebonite being made water- and air-tight with putty (P_1) below and plasticine (P_2) above. The floor of the tray sloped in all directions to an eccentrically placed, circular opening, O, on to the under side of which a short neck of stout brass tubing, N, was soldered. This neck was closed by insertion, from below, of a rubber bung, pushed in so far as to leave a small recess between its upper surface and the opening in the zinc tray. This bung had a central larger, and another smaller, perforation. Through the central hole passed a brass tube, 2 cm. in diameter, the lower end of which issued through a water-tight gland in the bottom of the tank. The upper end was closed by a thin rubber diaphragm, with a central perforation through which the long glass cannula, C, from the vena cava was pushed. The lower end of this cannula thus reached the exterior concentrically to the open end of the brass tube, without direct contact with the hot water in the tank. Through the other perforation in the bung, and ending flush with its upper surface, passed a short glass tube, D, connected by a rubber tube to another glass tube, D_1, which passed out through a gland in the lateral wall of the tank. This served as an accessory outflow tube, for the drainage of casual blood leaving the liver, other than that issuing by the vena cava and its cannula. The interior of the zinc tray, and of the brass neck above the rubber bung, was carefully coated with paraffin wax, so that even such small quantities of blood, as might thus escape from the main route of the perfusion, could make no contact with metal at any point. The lower end of the cava cannula was connected by rubber tubing to an outflow recorder, R, of the type described by Gaddum [1929], placed at such a height that the opening into it of the outflow tube was approximately on the same level as the stump of vena cava into which the cannula was tied. There was, accordingly, no resistance to the caval outflow, and no negative pressure which, by causing periodical collapse of the caval wall, might break the steady venous stream. The outflow recorder discharged into a funnel, F, which served as a venous reservoir, the outflow from its lower end to the oxygenator, OX, being restricted to a sufficient degree to keep a small reserve of blood always in the funnel. A light plug of cotton-wool in the bottom of the funnel acted as a filter for shreds of fibrin, or other solid particles which the blood might take up in passing through the liver. The accessory outflow tube, D_1, for casual blood or lymph, was also connected by rubber tubing to a short, bent glass tube, S, which hung over the edge of the collecting funnel. Before the sealing of the liver chamber, this accessory outflow path was filled with Ringer's solution to a level just above the upper surface of the bung, and its rubber tube connection was clamped. After the liver had been placed in position, its connections completed, the perfusion started, and the thermostat sealed, the clamp on the accessory outflow was removed, and the fluid in it allowed to adjust its level to that of the bend in the tube, S. Thereafter any addition to the fluid of this part of the system, by a small leakage from the liver, was automatically balanced by the drip of a corresponding volume from S, and no complication from this source affected the plethysmographic record.

The arterial and portal cannulæ were of the special shape, illustrated in Fig. 2, PC. The wide, vertical side tube on the bulb expansion allowed the cannula to be completely filled with blood, and was then closed by a small rubber cork, traversed by a small thermometer. Up to these bulbs the cannulæ were entirely outside the thermostat, and were fixed there by adjustable clamp-holders. The long beaks of the cannulæ, of which the nozzles were tied into the portal vein and hepatic artery, both entered the chamber through a wide common gap cut in the thick ebonite rim. This gap allowed freedom of adjustment of the cannulæ in three dimensions, and they were fixed in such positions that there was no tension on the vessels. The gap was then filled with fresh putty, which was readily moulded round the necks of the cannulæ, so that they passed air-tight through the mass. A similar gap cut in the opposite side of the rim, and also filled with putty, allowed a long, narrow glass cannula, the

nozzle of which was tied into the common bile duct, to lead away the bile, which was usually secreted steadily during the perfusion. This second gap also gave passage to the cava cannula when the goat's liver was perfused. The thoracic cava issues from the liver of the goat, to pass through the diaphragm, at a level nearer to the free border of the liver than in the carnivora. When the goat's liver is laid on its convex surface in the chamber, the cannula draining the cava lies naturally in a horizontal position, and passes out of the chamber at the side opposite to the entry of the inflow cannulæ. In the dog and the cat, on the other hand, the vena cava issues from a point near the middle of the convex surface; so that when the organ is laid in the chamber the cava opening is beneath, and the natural direction of drainage is vertically downwards, the brass tube and perforated rubber diaphragm, above described, being provided for its air-tight passage from the chamber in this direction.

Though the liver could be laid directly on the paraffined zinc tray, it was found that it could be more naturally disposed on a circular sling or hammock of mosquito net. For convenience of removal and cleaning, this net was attached, at its circumference, to the upper edge of a separate hoop of paraffined zinc plate, *M*, forming a short, wide cylinder, nearly equal in height to the vertical wall of the tray, and just sufficiently smaller in diameter to drop easily within it, and rest by its lower edge on the tray bottom. The net, *W*, was sufficiently slack to sag into a natural contour under the weight of the liver, but not so as to make contact with the tray. It has a wide, circular opening, bound with a strip of lawn to prevent fraying, and so placed as to be directly over the opening in the tray.

In most of our earlier experiments on dogs, oxygenation of the blood was effected by use of the lungs, rhythmically inflated by a Brodie pump, and perfused by a second Dale-Schuster pump. In such experiments one dog was completely bled under ether anæsthesia, the blood being whipped and filtered and used to fill the apparatus. The lungs were then prepared for perfusion and introduced in their proper place in the whole scheme, while the second dog, which supplied the liver, was being prepared. The second dog was also anæsthetized with ether, and a volume of blood, about two-thirds of the whole, was taken through a carotid cannula, about 200 c.c. of warm Ringer's solution being then injected by the jugular vein. This blood was whipped, filtered and added to that in the apparatus. The abdomen of the dog was then opened, the arteries to the intestines and stomach ligatured seriatim, and the lower end of the œsophagus doubly tied and cut. Cannulæ were then tied into the bile duct and the portal vein, the splenic and other tributaries to which were tied. The stomach and intestines were then removed, leaving the liver still supplied with natural circulation through the hepatic artery. A ligature was laid under the abdominal cava just below its entry into the liver, and above suprarenals. A sufficient dose of heparin to render the blood incoagulable was then given intravenously, and the remainder of the blood was allowed to run out through a clean carotid cannula. While this was in progress the final preparations for perfusion were completed. These included the tying of the cannula into the hepatic artery, the ligature of the abdominal cava, the opening of the thorax and tying of the drainage cannula into the thoracic cava close to the diaphragm, and the ligature of veins opening into the cava from the diaphragm. If the liver was to be placed in the thermostat for perfusion, it was than removed by cutting through the diaphragm. The liver was lowered on to the net in the chamber, the end of the long cava cannula being pushed through the rubber diaphragm provided for its passage, until its end could be seized below the tank. The accurate disposal of the liver, with its lobes in natural positions and the cava so placed in relation to the outflow cannula as not to be kinked, twisted or stretched, was given careful attention. The portal and arterial cannulæ were filled and connected to the appropriate parts of the perfusion scheme, and the cannula from the bile duct laid in position. Clips were then removed and the perfusion started. As soon as this was seen to be in satis-

factory progress, the cava cannula was connected to the outflow recorder, *R*, and the circulation was complete.

The next step was to close the thermostat chamber, which was also to serve as an air plethysmograph. For this we used the glass lid of a desiccator, with a central tubulus closed by a rubber cork, a tube through which served for connecting the air space by rubber tubing with a large bellows recorder. The seal between the ebonite rim and the glass lid was made with fresh putty. A thin roll of the putty was laid round the flat ebonite surface, and worked into continuity with the putty in the gaps transmitting the cannulæ. The warmed glass lid was then pressed down on it, and a perfect, air-tight seal was promptly obtained. This use of putty as a sealing agent, which we owe to our assistant Mr Collison, has given ease and security, in place of the trouble and uncertainty encountered when plasticine and vaseline were used, and we can strongly recommend it for similar purposes.

While the above procedure, using two animals, enabled the perfusion of the liver to be started with a minimal interval after stoppage of the natural circulation, and gave a good arterial tone in the earlier stage of the perfusion, it has not been necessary for all purposes. We have obtained good results by bleeding the animal completely under ether, with several intravenous injections of warm Ringer solution, totalling 200–300 c.c. in a large dog or goat, during the process, and then completing the dissection and fixation of the cannulæ after circulation had ceased. In some cases we washed the liver vessels out with warm Ringer solution before transferring the organ to the thermostat; but, in all cases when heparin was not used, we caught the first 100 c.c. or so of blood before completing the circuit, and re-whipped and filtered this portion before returning it to the system. When this simplified procedure was adopted we used an artificial oxygenator of the type described by Hooker [1915], in which the blood is spun from a rotating disc on to the inner surface of a wide cylinder, through which oxygen passes. To prevent undue loss of water from the blood, the oxygen was first bubbled through physiological saline, in a bottle immersed in the warming bath, *B*. For simplicity this is omitted in Fig. 1. A cylinder of appropriate length and width, to oxygenate the rapid venous stream from the liver of a dog or goat, was made from sheet celluloid. The lower end of this cylinder rested in the inverted dome-lid from a large desiccator, *A*, in which the oxygenated blood collected, for withdrawal to the pump and portal reservoir. An artificial oxygenator of this type was used for all experiments on the goat and the cat, since the lungs of these animals readily become œdematous under perfusion.

It was possible to arrange for stimulation of the hepatic plexus, dissected from the hepatic artery before insertion of the cannula, with the liver in the plethysmograph, the electrodes passing, with the cannulæ, through the putty filling the gap in the ebonite rim of the chamber. For stimulation of the splanchnic nerves another arrangement had to be made, in which the chamber was eliminated. For this purpose the hepatic artery was carefully dissected out of the sheath of nerves, which were left uninjured. The splanchnic nerves were dissected, tied, and laid ready for stimulation. The other preparations were made as usual, except that the diaphragm was left intact and the liver in position. The body was then doubly transected, first through opposite lower intercostal spaces and the corresponding levels of sternum and thoracic vertebræ, and second through the abdominal wall and spinal column just above the kidneys. The segment being then turned with the thoracic side downwards, the diaphragm formed a natural sling for the support of the liver, and drainage from the vena cava was performed from below. The preparation was supported by skewers passing through the body wall and resting on a retort ring. The liver was kept warm, during the relatively short perfusion, by wet cloths, frequently renewed; but no volume record was possible in this case.

III. RESULTS.

We may mention first certain general features of the artificial perfusion of the livers of the different animals used—dog, goat and cat. Our experiments, being made with pressures artificially adjusted, give no direct evidence concerning the relative contributions to the liver circulation of the arterial and portal inflows under natural conditions. The rate and stroke volume of the arterial pump could only be adjusted to give what we judged, from the arterial pressure record, to be a rate of flow within normal limits. Provided that the liver was still in good condition, and the portal inflow accordingly free, we found, in fact, that this adjustment gave us a similar contribution of the arterial to the total flow, in the dog, to those recorded *in vivo* by earlier observers, viz. about 25–30 p.c. The same probably holds for the cat, in which the size of the hepatic artery has about the same relations to those of the portal vein and the liver as those seen in the dog. In the goat, on the other hand, the hepatic artery is relatively much smaller, and we found that, under similar conditions of empirical adjustment, the artery was furnishing only 15–20 p.c. of the total flow through the liver of that animal.

It was easy with our perfusion system to demonstrate the existence of a partially reciprocal relationship between the two inflows. If the arterial inflow was reduced by compression of the rubber connection between the pump and the cannula, or by reducing the stroke of the pump, the portal inflow was accelerated. The reciprocity, however, was not complete; any reduction of arterial inflow produced some reduction of total outflow and of liver volume. To a smaller extent the resistance to the arterial inflow, as shown by the pressure produced with constant pump stroke, was increased by accelerating the portal inflow by raising the portal reservoir, and decreased by lowering the reservoir, or by constricting the rubber connection to the portal cannula. It is unlikely, however, that these relatively small changes of resistance to the arterial flow, due to changes in the pressure in the lobular sinusoids, were sufficient to modify significantly the input by the hepatic artery. The pump is not rigid, but its output is not proportionately affected by small changes of resitance. The portal flow was, in any case, far more sensitive to changes directly affecting the arterial flow than the latter was to primarily portal changes; and this is almost certainly true under natural conditions, as well as in those of our experiments. The existence of this partial reciprocity, or vicarious function, of the two blood supplies could be demonstrated in another way. The total outflow being determined, with both inflows in function, each

could be in turn shut off, and, after waiting in each case for the liver to assume a new equilibrium volume, the outflow produced by each supply, acting separately, could be recorded. The sum of these two independent flows through the liver was always substantially greater than that produced by the combined action of the two supplies, with the same adjustment of pump and reservoir throughout.

While, with the exceptions above mentioned, the conditions of the circulation in the liver of the dog, the cat and the goat were similar at the beginning of a successful perfusion, they did not long remain so. In the cat and the goat the artificial circulation, when once successfully started, continued for periods of several hours without serious alteration. The outflow from the vena cava continued free, and, indeed, usually showed a slow, progressive acceleration as the experiment proceeded. The liver also remained relatively normal in appearance, and showed no rapid increase in volume. In the dog, on the other hand, at a varying interval after the start of the perfusion, but usually within the first 30 min., the outflow from the vena cava gradually became less, while the liver volume steadily increased, and the organ became dark in colour and congested in appearance. A secondary equilibrium was usually attained, in which the outflow and liver volume became relatively constant, apart from changes produced by drugs or nerve stimulation. It was obvious, however, that there was some factor complicating the maintenance of the artificial circulation in the dog's liver, which was absent, or much less effective, in the case of the cat and the goat. It will be convenient, accordingly, to describe separately the experiments on the dog's liver.

Experiments on the liver of the dog.

As we have already indicated, the conditions of the blood flow in the perfused liver of the dog are complex and variable, and often show an important modification during the course of an experiment. If the interval between the stoppage of the natural arterial flow and the beginning of the perfusion is reduced to a minimum—10–15 min.—the initial stages of the perfusion often show a gradual development of arterial resistance, so that the arterial pressure rises to, and maintains for 10–20 min., a "normal" level of 150 mm. or more of mercury. At this stage the effects of vaso-dilator drugs on the arterial resistance can be shown with special clearness. During this rise of arterial resistance the flow in the portal vein usually shows a corresponding acceleration, as indicated by a gradual fall in the lateral pressure in the tube leading to the portal cannula. As the arterial pressure subsides again, the pressure in the

portal vein usually rises, and then continues, throughout the experiment, gradually to rise. In the early stages the effect is, doubtless, due to the partial compensation of the one flow by the other. As the rising arterial tone shuts off more effectively, from the lobular plexus, the pressure created by the pump, the resistance to the portal flow diminishes, and increases again as the arterioles relax. After the arterial pressure has sunk to a steady level, however, the resistance to the portal flow usually continues to increase. We could have attributed this to an increasing tone of the portal branches, but for the fact that it was accompanied by swelling of the liver, as well as diminishing outflow. In extreme cases the level of the blood in the portal manometer has eventually risen slightly above that in the constant-level reservoir supplying the portal vein, showing that there was actually, at this stage, a reverse flow from the liver, out through the portal vein, and up into the reservoir. This could only mean that a resistance had developed, to the outflow from the lobular capillaries, sufficiently strong to prevent blood leaving them as fast as it entered them from the arteries alone, so that the pressure in the lobules rose until it became higher than the level in the portal reservoir.

This resistance to the outflow from the lobular capillaries, shown thus in extreme form, appears to be characteristic of the dog's liver, and introduces a complication into the interpretation of vascular changes in that organ. Since it practically always becomes more pronounced as the perfusion proceeds, and as the tone in the branches of the artery and portal vein subsides, it is obvious that the resultant effect of a drug, or of nervous impulses, affecting the resistances on both sides of the capillary bed, will be likely to change during the course of an experiment.

Adrenaline. It is easy to demonstrate the facts, already well known from earlier publications, that adrenaline causes constriction of the branches of both hepatic artery and portal vein. Early in the perfusion, when the tone of the arteries is good, and the portal inflow free, a rise of arterial pressure, and a rise of lateral portal pressure indicating a slower portal inflow, may be the most prominent effects of a small dose (5–20 γ) of adrenaline. The two naturally differ in prominence according to the route by which the adrenaline is injected. If it is injected into the arterial cannula, the result is a relatively large rise of arterial pressure, and a small rise of portal pressure. The latter is often succeeded by a fall, indicating accelerated portal inflow. Since artificial diminution of the arterial flow causes some fall of the portal pressure, this secondary fall might well be interpreted as due to persistence of the arterial constriction after the direct effect of adrenaline on the portal branches has subsided.

Such an effect is certainly involved, but we shall see that another plays a more important part. If the injection is made into the portal cannula, the rise of portal pressure is correspondingly large, and that of the arterial pressure small, or even insignificant.

Our attention was early drawn to the fact that, in spite of these arterial and portal constrictions, both tending to lessen the inflow into the liver, the outflow from the vena cava usually showed a definite increase, as the result of injecting a small dose of adrenaline, into either the portal vein or the hepatic artery. This effect was variable, especially in the earlier stages of the perfusion. In some cases a relatively large dose of adrenaline, causing a pronounced constriction of both arterial and portal branches, would be accompanied by a diminished outflow, while a subsequent smaller dose, producing a small rise of arterial pressure, would cause a definite increase of venous outflow, with a small initial rise followed by a more pronounced fall of portal pressure. Such contrasted effects, of two successive injections of adrenaline of different dimensions, is illustrated in Fig. 3. In this and other similar figures, the lines of tracing from above downwards show (1) the rate of venous outflow, the height of each line showing the volume of blood leaving the liver in a constant time unit, and therefore the rapidity of the flow; (2) the volume of the liver, *L.V.*, by a large bellows recorder attached to the plethysmograph; (3) the lateral pressure, *P.P.*, in the portal cannula, moving inversely with the rate of portal inflow; and (4) the arterial pressure produced by the pump. It will be seen, in Fig. 3*a*, that a dose of 20γ of adrenaline, injected into the hepatic artery, causes a large rise of arterial pressure, a moderate rise of the portal pressure, a diminution of the liver volume, and a diminution of the rate of outflow. There is nothing here to suggest anything but straightforward vaso-constrictor effects on arterial and portal branches. The next section, *b*, of the tracing shows the effect of an immediately following injection of only one-tenth the amount of adrenaline—2γ. There is a correspondingly small rise of arterial pressure. The portal pressure shows an initial small rise (retardation) followed by a much more pronounced fall (acceleration) The effect on the liver volume, though relatively evanescent, shows at its maximum a larger shrinkage of the volume than that caused by the previous larger dose. The effect on the outflow is a pure acceleration.

When we first observed this effect on the outflow we tried to explain it as due to a general contraction of the arterial tree, exercising a squeezing effect on the lobules. If the effect were of that kind, a dose producing a strong arterial constriction, like that in Fig. 3*a*, should exhibit the effect

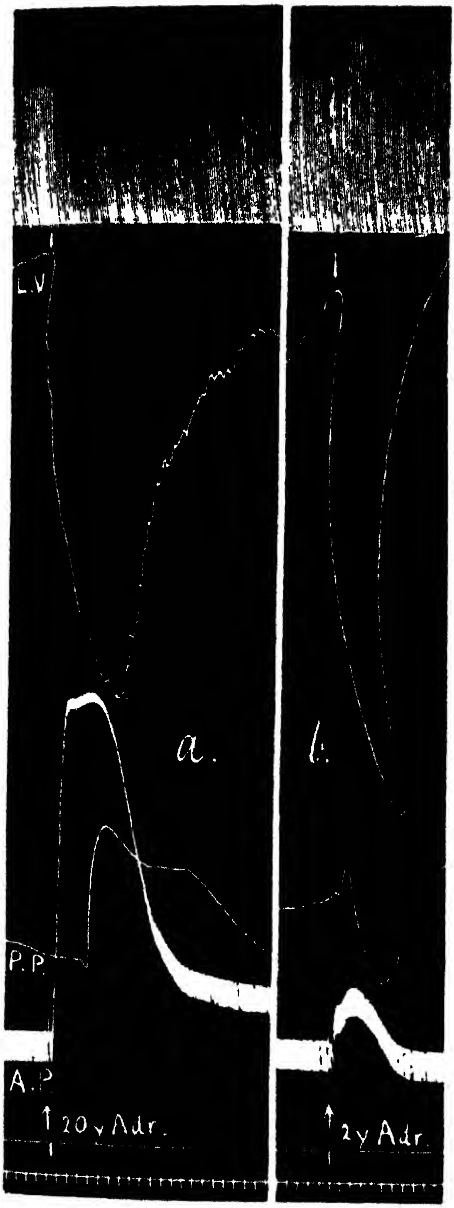

Fig. 3 *a*, *b*. Dog's liver. Different doses of adrenaline.

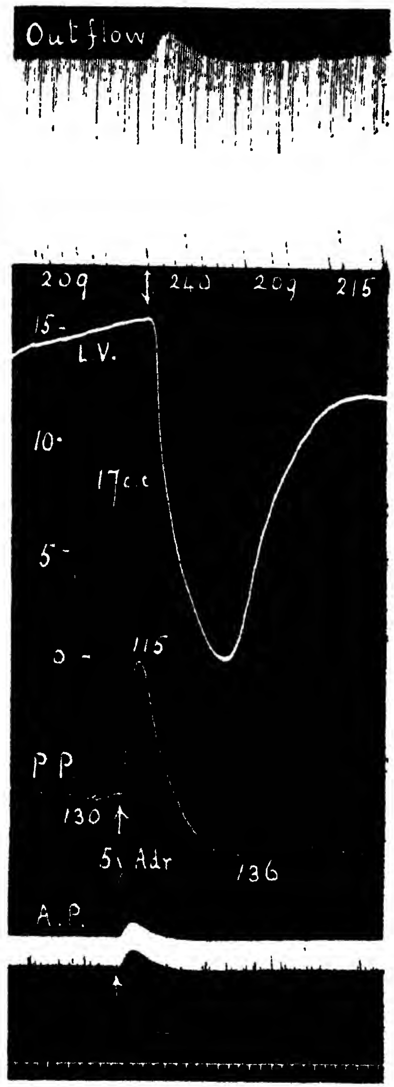

Fig. 4. Dog's liver. Portal injection of adrenaline.

more strongly; whereas it is most regularly produced by smaller doses, causing relatively little arterial constriction, as in Fig. 3*b*. The effect should, further, be absent or insignificant when the injection is made into the portal vein, and the action on arterial tone correspondingly small. That this is not the case is shown in Fig. 4. This tracing is one of a series from one experiment, in which the different recording systems were carefully calibrated; the figures written in relation to the records of outflow and of portal pressure, *P.P.*, representing, in each case, c.c. per min. It will be seen, in Fig. 4, that 5γ of adrenaline injected into the portal vein causes a very small rise of arterial pressure, a decrease of portal inflow from 130 to 115 c.c. per min., a decrease of liver volume amounting at its maximum to 17 c.c., and an increase of outflow from 209 to a maximal rate of 240 per min. Leaving out of account the small change in arterial tone, which probably had practically no effect on the output of the pump, we have, at the maxima of the effects, a diminution of inflow by 15 c.c. per min., an increase of outflow by 31 c.c. per min., and a loss of liver volume, on balance, of 17 c.c. Such an effect seemed to be intelligible only on the supposition that there was some resistance to the venous outflow which a small dose of adrenaline directly reduced. It appeared probable, further, that the temporary reduction of this resistance, with consequent fall in the pressure in the lobular capillaries, was the principal factor in the secondary acceleration of portal inflow, as seen in Fig. 3*b* and in Fig. 5. This latter record, taken from a later stage of the same experiment as Fig. 4, shows the effect of a small dose of histamine, to which further reference is made later, and then of three successive arterial injections of adrenaline, the last being only 1γ, a dose which may probably be regarded as within strictly physiological limits. It will be seen that the portal inflow undergoes, in the three cases, maximal accelerations of 16, 15 and 6 c.c. respectively. The corresponding maximal increases of outflow are 50, 50 and 27 c.c. The fall of portal pressure cannot be secondary to the arterial constriction; if it were, the outflow would not be thereby accelerated, since reduction of arterial is never even completely compensated by increase of portal flow. It cannot be due to relaxation of the portal branches; if it were, the increased outflow would be accompanied by swelling of the liver, instead of by large shrinkage. The relaxation of a dominant resistance on the outflow side of the capillary bed is the only possibility.

It still seemed just possible, though very unlikely, that some kind of turgescence, caused by relaxation of arterial tone, had produced a compression of the efferent veins, and that the relief of this pressure by

arterial constriction allowed the freer outflow. This possibility could be excluded in two ways.

(1) After a suitable dose of ergotoxine has been given, adrenaline causes not constriction, but further relaxation of the arterioles, with fall of the arterial blood-pressure. Ergotoxine similarly annuls the constrictor

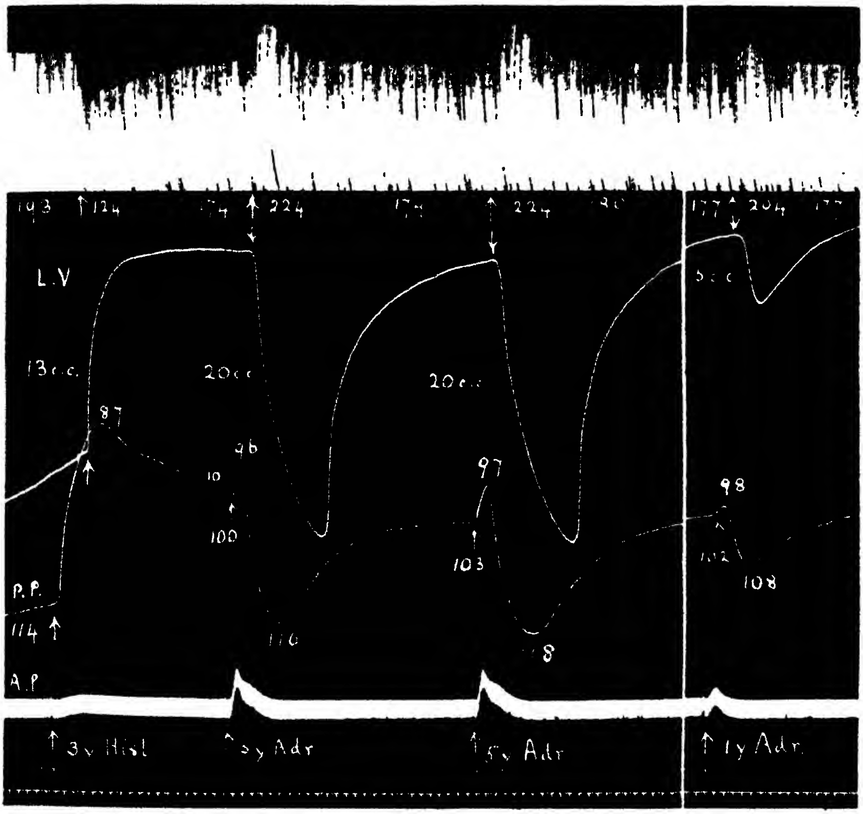

Fig. 5. Same experiment as Fig. 4. Successive arterial injections of histamine and adrenaline (thrice).

effect of adrenaline on the portal branches. When adrenaline is injected after ergotoxine, we accordingly obtain an uncomplicated increase of inflow. If arterial constriction were in any way concerned with reducing the resistance to outflow, this effect should be absent after ergotoxine, and any increase of outflow then produced by adrenaline should be not greater than the increased inflow. Fig. 6 shows that it is, in fact, much

greater; there is a very large increase of outflow accompanied, in spite of the increased inflow, by the usual decrease of liver volume. Accurate calibration figures are not available for this experiment, but the outflow at its maximum was greater by 54 p.c. than before the injection of adrenaline.

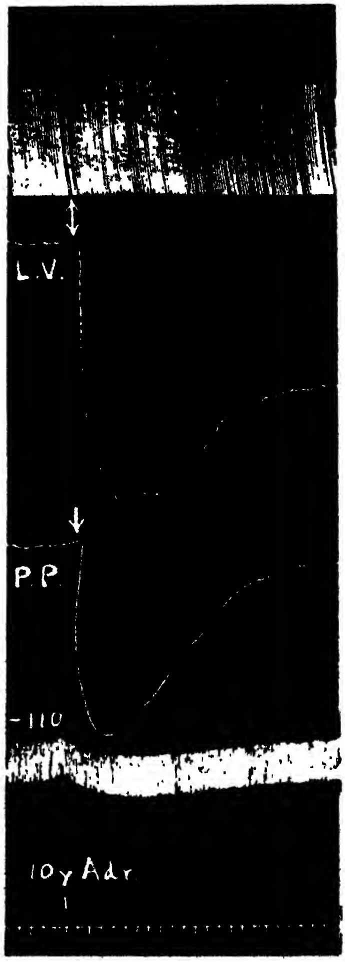

Fig. 6. Dog's liver. Adrenaline after ergotoxine.

(2) If adrenaline, instead of being injected into the blood going direct to artery or portal vein, is added in relatively large dose to the vessel receiving the blood from the liver, so as to become mixed with nearly the whole of the blood in the system before it reaches the liver vessels, it has very little effect on the tone of arterial and portal branches, but may have a very great accelerating effect on outflow and on portal inflow. Fig. 7 is a record from an experiment of this kind, in which a strong outflow resistance had developed early in the perfusion, with consequent slow flow through a swollen liver. Volume and portal pressure are not reproduced in this figure; the figures just above the base line give the rates of portal inflow calculated from the tracing. It will be seen that, until the adrenaline reached the liver, total outflow and portal inflow were still falling, from 40 to 37 and 25 to 23 c.c. per min. respectively; the difference of 14–15 c.c. represents the arterial inflow. At the mark on the base line 100γ of adrenaline were added to the issuing blood, and when this had passed through the oxygenator, become mixed with the reserve, and reached the liver, the effect shown in the second section of the tracing began. It will be seen that the outflow rose rapidly to 112 c.c. (200 p.c. increase) while the portal inflow rose to 50 c.c. The difference of 62 c.c. cannot, in this case, be attributed to arterial flow, since the liver volume suffered a decrease far beyond the recording range of the large bellows employed, so that a large part of the excess outflow was due to depletion of the liver. The fact that little

change of arterial pressure occurred with constant pump stroke may be taken to indicate that the arterial contribution was practically unchanged. It will be seen that 24 min. after addition of adrenaline to the blood, the outflow was still slightly greater than before its action began.

It will be clear that, with adrenaline acting at three different points of the vascular system, constricting the branches of the hepatic artery and portal vein, and relaxing a tonic resistance somewhere on the outflow side of the capillary plexus, varying results may be expected, with different doses and different states of relative tone in the three types of vessels

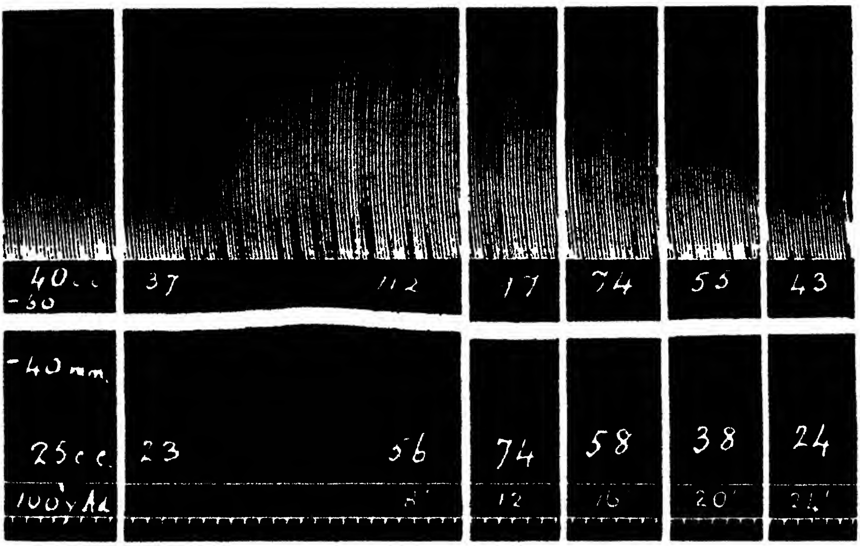

Fig. 7. Dog's liver. Adrenaline mixed with general volume of blood.

concerned. The variety observed was, indeed, great; and we can only illustrate a few types of response. One additional complication should be mentioned, as revealed by a closer study of Fig. 3. Attention has already been drawn to the greater amplitude of the volume reduction caused by the second dose of 2γ, than by the first dose of 20γ of adrenaline. The fact that the larger dose causes a diminution, the smaller an increase of outflow, is partly accounted for by the restricted inflow which the larger causes. It cannot be wholly due to this, however. If the larger dose had weakened the resistance to outflow as much as the smaller, then the combination of this effect with restricted inflow must have caused the liver to empty more completely with the larger dose. The fact that, in spite of

restricted inflow, it lost l e s s volume, strongly suggests that 20γ of adrenaline, in this instance, produced a smaller reduction, or even some increase of the resistance to outflow. This is only one example of records which suggested a double mechanism of control on the outflow side of the capillaries, responding by relaxation to small and by constriction to larger doses of adrenaline. We shall return to the point again in considering the localization of the control.

Sympathetic nerve supply. We have obtained results of the same general type as those produced by adrenaline, by stimulating the plexus of nerves

Fig. 8. Dog's liver. Stimulation of right splanchnic nerve.

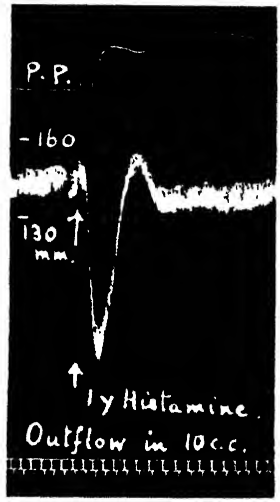

Fig. 10. Dog's liver, early in perfusion. Small arterial injection of histamine.

accompanying the hepatic artery, or either the right or left splanchnic nerve. Fibres of vagus origin must be assumed to run with the sympathetic fibres in the hepatic nerve bundles, since vagus stimulation affects the tone of the gall bladder [Bainbridge and Dale, 1905]. If, however, the vagus fibres have any effect on the liver blood vessels, they have not retained it under the conditions of our perfusion. Figs. 8 and 9 illustrate effects of stimulating the sympathetic nerve supply. Fig. 8 shows the effect of stimulating the right splanchnic nerve. It is from an early experiment, in which the outflow was recorded by a tilting bucket, each mark, on the line just above the time record, signalling the discharge of 10 c.c. The top line records the lateral pressure in the portal cannula. It will be seen that stimulation of the nerve causes rise of arterial pressure,

increased portal inflow, and acceleration of outflow. Fig. 9 gives a more complete record, the liver being enclosed in the plethysmograph and the hepatic nerve bundles stimulated. The outflow had become very much restricted, the liver congested, and the portal inflow reduced to only 2 c.c. per min. Stimulation of the hepatic nerves produces an effect closely resembling that of adrenaline under such conditions—increase of the outflow from 19 to 55 c.c. per min., increase of portal flow from 2 to 23 c.c., decrease of liver volume, and constriction of arterioles. It is to be noted that the effects on outflow, portal flow and volume long outlast that on the arteries. Clearly the mechanism restricting the outflow is inhibited by sympathetic stimulation, as by small doses of adrenaline.

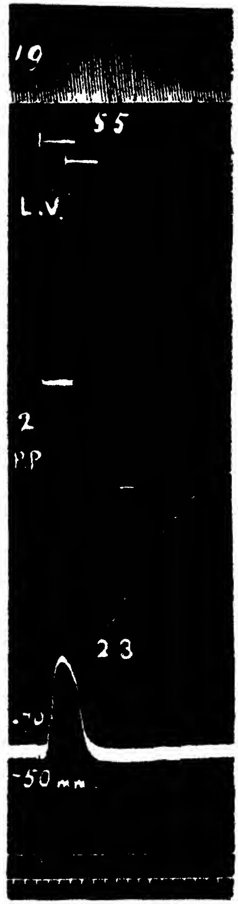

Fig. 9. Dog's liver. Stimulation of hepatic nerves.

Histamine. At any early stage of the perfusion, when the arterial tone is high, a very small dose of histamine produces its characteristic depressor effect on the arterial pressure. Fig. 10, from an early experiment, in which the volume of the liver was not recorded, and the outflow was registered by the 10 c.c. tilter, shows this effect, as produced by an arterial injection of 1γ of histamine. The portal pressure shows an initial rise corresponding to the arterial depression. It is probably due in part to the relaxation of arterial tone, but also in part to weak constriction of portal branches, since a portal injection produces a more pronounced effect of this kind. It will be seen that there ensues a second, longer rise of the portal pressure, accompanied by a definite restriction of the outflow. This is probably an early indication of what later, in practically all experiments, becomes the predominant effect of histamine. It will be remembered that it was the action of histamine, in causing swelling of the dog's liver, which first drew the attention of Mautner and Pick [1915], followed by other workers in the Vienna school, to the contractile function of the hepatic veins. The first record in Fig. 5 shows a typical effect of histamine (3γ) on the dog's liver. The conspicuous features are the reduction of outflow, swelling of

the liver, and rise of pressure in the portal manometer, indicating diminished inflow. It will be seen that the effect, at its maximum, reduces the outflow by 69 c.c. per min., the portal inflow by 27 c.c. The arterioles have lost their tone and cannot further relax; the trifling rise in arterial pressure may well be due to the increased resistance to outflow. The effect of histamine, then, on the outflow control, as Fig. 5 well illustrates, is the opposite to that of adrenaline; it increases the resistance which adrenaline relaxes. In a liver with well-developed tone of the resistance to outflow, but well-maintained perfusion, such as that which gave Fig. 5, small doses of histamine and adrenaline can be alternated almost indefinitely, histamine always causing a restriction of outflow and swelling of the liver, while the succeeding dose of adrenaline opens the sluice, lets the accumulated blood escape, and restores the former condition. These effects of histamine and adrenaline are unchanged by ergotoxine.

Acetylcholine. Having failed to obtain any clear evidence of vagus control of the liver circulation, we did not expect to get effects of any importance with acetylcholine. The results, indeed, produced by injections of up to 20γ of this substance into artery or portal vein, were always small, and in many cases almost imperceptible. If the arterial tone was high, an arterial injection of acetylcholine produced a relaxation, comparable to that produced by histamine in Fig. 10, but smaller. When any effect was produced on the portal flow, it was in the direction of retardation. Fig. 11, from the same experiment as Figs. 4 and 5, shows the one case in which we obtained what appears to be a clear restriction of outflow with acetylcholine. The injection, of 10γ, was made by the artery, and the prompt and rapid rise of portal pressure, indicating a decrease of inflow by 13 c.c. per min. at the maximum, suggests a direct increase of tone of the portal branches. The outflow, however, falls at the same time by 47 c.c. per min., and the liver volume increases, though only by about 3 or 4 c.c. There is certainly some increased resistance to outflow, and it is not possible to be certain whether the rise of portal pressure is entirely due to this, or involves some constriction of portal branches in addition. The arterioles had lost their tone, and the arterial pressure shows a slight increase. We record this effect, as being the only case in which, either with vagus stimulation or acetylcholine, we have obtained anything which might appear comparable with the closure of the hepatic veins in response to vagus stimulation, which Mautner claimed to have observed by a special method. The effect of atropine in increasing the rate of outflow, described by Grab, Janssen and Rein [1929*a*], also suggests a control of

the outflow resistance by the vagus, which the conditions of our experiments did not enable us clearly to demonstrate.

Nature and location of the resistance to outflow. The workers of the Vienna school, who described the production of the "Lebersperre" by

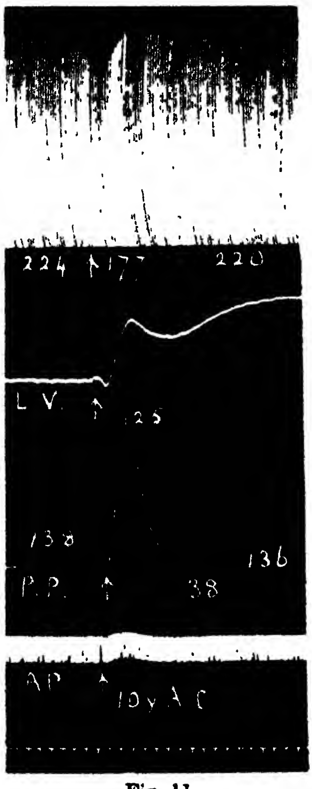

Fig. 11.

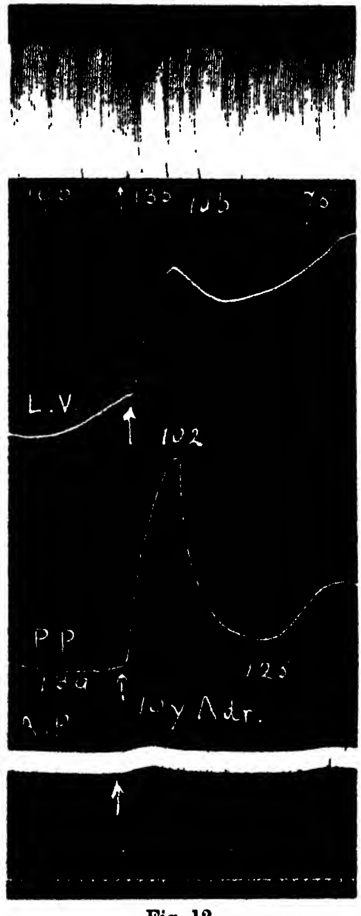

Fig. 12.

Fig. 11. Same experiment as Figs. 4 and 5. Effect of acetylcholine.

Fig. 12. Same experiment as Figs. 4 and 5, after removal of vena cava and ends of main hepatic veins. Portal injection of adrenaline.

histamine, have been inclined to locate the effect in the smaller tributaries of the hepatic veins. A study of the anatomical evidence, however, which will be mentioned later, suggested to us that the obstruction might be located in the largest veins, at or near to their opening into the vena cava.

In several experiments we put this possibility to the practical test. Having obtained a liver in which the resistance to outflow, with intensification by histamine and reduction by adrenaline, was clearly demonstrated, we stopped the perfusion, removed the liver from the thermostat, slit open the intra-hepatic cava completely, and cut away the whole of its wall, with the mouths of the hepatic veins, and the liver substance adjoining the cava to a depth of about 5 mm. The cava cannula was, of course, removed, and the opening in the rubber diaphragm through which it had passed from the chamber was sealed with a glass plug. The liver being again placed in position on the net sling, all blood flowing from it now passed out by the accessory outflow tube DD_1, which was now connected to the outflow recorder.

On resuming the perfusion after this mutilation, we obtained a surprisingly uniform, though somewhat freer outflow than with normal drainage from the vena cava. The effects produced by adrenaline and by histamine were now, in all cases, quite different from those which they had produced with the caval ends of the hepatic veins intact. Fig. 12 is taken from the experiment which, before the secondary operation, gave Figs. 4, 5 and 11. It shows the effect now produced by portal injection of 10γ of adrenaline. It will be seen that the portal inflow is lessened by 37 c.c., the outflow by 61 c.c., while the liver volume increases by about 7 c.c. Adrenaline has probably, as before, produced some increase of tone of the portal branches. It has certainly produced an increased resistance to the outflow, in place of the diminution seen earlier. We seem here to have clearly demonstrated the increase of resistance to outflow which, in discussing Fig. 3, we found reason to suspect, as occasionally appearing in the action of larger doses of adrenaline on the intact liver. This, presumably, is the mechanism which, in Baer and Roessler's [1926] reversed perfusion of the liver from the vena cava, produced an increase of resistance to the inflow when adrenaline was added to the perfusion fluid. It is also probably responsible for the swelling of the liver in the living dog, produced by relatively enormous doses of adrenaline, in the experiments of Bainbridge and Trevan [1917] and of Lamson [1915–21]. In continuation of the experiment after Fig. 12, small doses of histamine, such as had earlier produced such pronounced restriction of outflow and swelling of the liver, were without perceptible action. A much larger dose (60γ) retarded the portal inflow by 12 c.c., the outflow by only 17 c.c., and caused a small increase of liver volume. Histamine has, therefore, a weak remnant of augmentor action on the deeper seated resistance to outflow, which adrenaline more strongly augments. This deeper effect could not

be demonstrated in all our experiments of this kind. It is presumably a contraction of hepatic veins, smaller than the main veins opening directly into the vena cava. In these latter, it appears, the main resistance is located, constituting a kind of sphincter which is intensely stimulated by histamine, widely opened by adrenaline, in what may be regarded as physiological doses.

Fig. 13 is taken from another experiment, in which the first attempt to remove the sphincter mechanism, by cutting through the liver substance as closely as possible to the wall of the cava, failed of its purpose. The

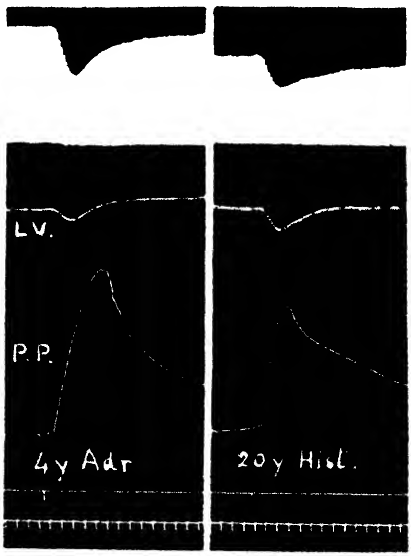

Fig. 13. Dog's liver after slitting up hepatic veins. Portal perfusion only. Effects of adrenaline and histamine.

perfusion was being conducted through the portal vein only, and successive doses of adrenaline still regularly caused an increase of portal inflow (fall of pressure) and an increase of outflow from the hepatic veins; while histamine diminished both inflow and outflow, and caused some swelling of the liver. The liver was again removed, and each visible hepatic vein was slit up for about 0·5 cm., one blade of the scissors being inserted into its lumen. The perfusion was then resumed, and adrenaline and histamine now produced the effects shown in Fig. 13. It will be seen that the effects of both are now preponderantly on the tone of the portal branches; the portal pressure rises, the outflow diminishes, and the liver shows a small shrinkage. It is not possible to exclude a minor effect on resistance to

outflow; indeed, the relatively small change in liver volume suggests that this resistance, too, may in both cases be somewhat increased. The main resistance to outflow, however, which previously dominated the control of perfusion rate, and was affected in opposite senses by adrenaline and histamine, has been removed. It will further be noted that a much larger dose (20γ) of histamine is now required to produce an outflow reduction comparable to that produced by 4γ of adrenaline.

The evidence, accordingly, seems to be conclusive in favour of a sphincter mechanism, located near the caval openings of the hepatic veins of the dog, the tone of which is intensely augmented by small doses of histamine, and relaxed by small doses of adrenaline and by sympathetic nerve impulses. There is evidence, though less complete, that the constrictor effect of histamine extends, with diminishing effect, on to the deeper part of the hepatic veins and their branches, and that in this region adrenaline also has a relatively weak constrictor action. These actions on the sphincter or sluice mechanism of the dog's liver, together with the chemical and nervous control of the double inflow, suffice to explain the complex and, at first sight, bewildering variety of changes in the circulation through that organ, in response to different stimuli.

Experiments on the livers of the cat and the goat.

As already mentioned, there is no evidence in the liver of these other species of a resistance to outflow, developing during the progress of perfusion, as in the dog. The outflow appears to remain perfectly free, and to become quicker rather than slower over a period of hours. In correspondence with this, we can find practically no evidence of any influence of adrenaline, histamine or acetylcholine on the resistance to outflow from these livers. Figs. 14 and 15 show typical effects of adrenaline and histamine on the cat's and goat's liver respectively, under conditions of perfusion and record identical with those used for the dog's liver. It will be seen that in the cat the restriction of portal inflow caused by adrenaline is relatively large, that caused by histamine so trifling that it can be regarded as a secondary result of the diminished resistance to arterial inflow. The two small changes, indeed, so balance one another that the volume of the liver is not perceptibly affected. The plethysmograph, it will be seen, was recording a slowly rising volume, the course of which was unchanged by the injection of histamine. In the goat the reverse relation appears to hold, the portal tone being little affected by adrenaline, and relatively sensitive to an augmentor effect of histamine. The changes in the volume of the liver and on the rate of outflow are, in all cases, those

which would be expected from the changes produced in the resistances to the portal and arterial inflows. There is nothing to suggest a variable outflow resistance. In only one experiment on the goat did we detect a relatively small increase of resistance to outflow as the result of injecting histamine by the artery; but in that experiment the effect was regularly

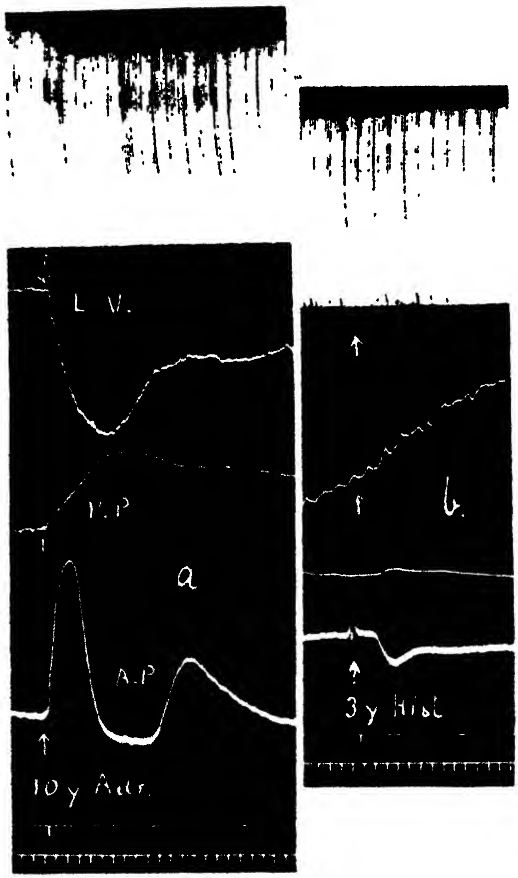

Fig. 14. Cat's liver. Effects of adrenaline and histamine.

repeated. With acetylcholine we obtained, in the cat's liver, only the expected fall of arterial tone. In the goat's liver it produced an effect which is worthy of separate mention. Relatively small doses (10γ), injected by the arterial route, caused effects weaker than, though generally similar to, those produced by adrenaline (Fig. 15c). Arterial and portal tone were increased, liver volume diminished, and outflow practi-

cally unchanged. The fact that the outflow was, if at all affected, slightly accelerated, in spite of arterial and portal constriction, suggests a diminution of resistance to outflow, but the effect was too small to be detected with certainty. This effect of acetylcholine was of the parasympathetic type, in that it was completely abolished by atropine, which left those of

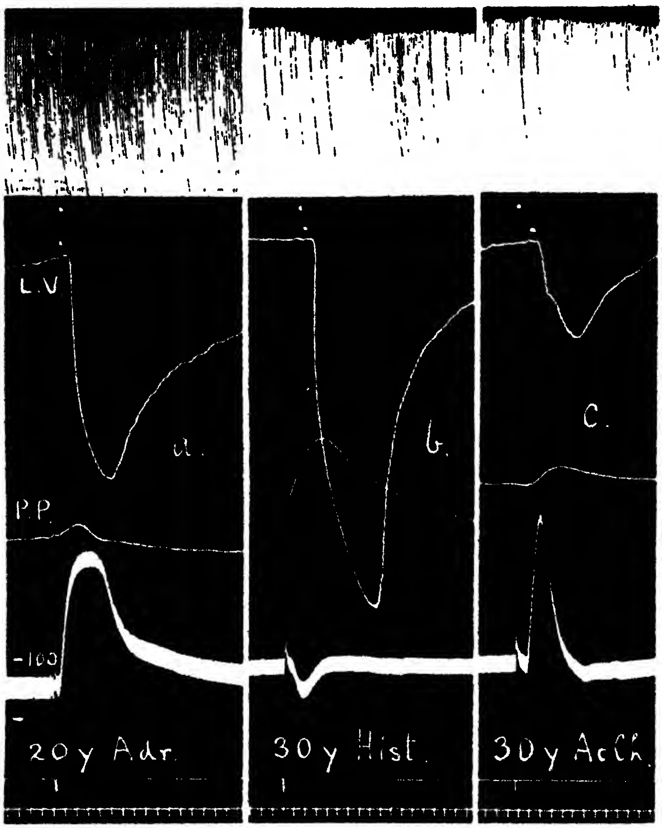

Fig. 15. Goat's liver. Effects of adrenaline, histamine and acetylcholine.

adrenaline and histamine unchanged. It is of interest to contrast this effect of acetylcholine on the hepatic arterioles with the distinct depressor effect of histamine (Fig. 15*b*). Experiments hitherto unpublished have shown that acetylcholine has its typical dilator effect on the arterioles in other parts of the goat, such as the limb. It may be noted that Daly and v. Euler [1932] have recently described a constrictor effect of acetylcholine on the branches of the dog's pulmonary artery, also abolished by atropine.

Note on the anatomy of the hepatic "sphincter."

The behaviour of the dog's liver under perfusion, and in response to chemical and nervous stimuli, is so remarkably different from that of other species hitherto studied, that a clear anatomical basis for the difference might be expected. We should expect to find clear evidence of a strong development of a muscular coat in the caval ends of the dog's hepatic veins, lacking in other animals. Hitherto, however, the anatomical evidence, as to the nature of the mechanism producing the variable resistance to outflow, is far less convincing than the physiological evidence of its existence. Simonds [1923], indeed, devoted special attention to the hepatic veins of the dog, with the object of discovering an anatomical basis for reactions such as Mautner and Pick [1915] had described. A paper by Arey and Simonds [1920] states that the hepatic veins of the dog have "an enormous amount of smooth muscle in the wall, thus demonstrating an adequate anatomical basis for impeded vascular flow should spasm occur." In a second note Simonds [1923] claims to have investigated twenty-two different species, and to have found more muscle in the hepatic veins of the dog than in those of any other. Unfortunately, the details as to the species examined and as to the distribution of the muscle coat in different parts of the hepatic veins, are not sufficient to enable us to link up Simonds' description directly with our own physiological findings.

Other more detailed descriptions relate to the hepatic veins of man. Elias and Feller [1926] state that, when hot formaldehyde solution (70–80°) is injected by the vena cava into a liver freshly obtained from the human cadaver, a constriction appears at the caval end of each hepatic vein. This is shown, in section, to be due to a plain muscle coat, increasing in thickness towards the opening of the vein, and blending with the relatively thick muscle coat of the vena cava. In a contribution to a recent discussion Elias and Feller [1931] state that the constriction takes the form of crescent-shaped projections into the lumina of the hepatic veins near their openings, seen in section as spur-like processes. The formation of these obstructive folds can be induced by barium chloride or pituitary extract as well as by heat. Miyake [1929] also states that the hepatic veins of man, in contrast to the portal branches, are equipped with a rather thick coat of longitudinally disposed muscle fibre, similar to that of the hepatic cava.

Our own physiological experiments, designed to remove the resistance to outflow in the dog, were stimulated by Elias and Feller's description.

We also, repeating their procedure, injected the dog's liver with hot formaldehyde solution from the cava and obtained a similar appearance of constriction at the hepatic outlets. So far as our observations have gone, we have found a similar arrangement of muscle coats in the hepatic veins and cava of the dog to that described by others for man. The hepatic veins have strong muscular coats, thickening towards their orifices; but the general direction of the fibres seems longitudinal, they appear to be continuous with those of the cava, and we have seen nothing to suggest an actual sphincter with transversely disposed fibres. In the cat the general arrangement appears similar to that in the dog, but the muscular coats in the hepatic veins and hepatic cava are much less strongly developed. The rich development of plain muscle in the dog's cava comes to an end as the vein passes out of the liver and through the diaphragm. In the goat the hepatic portion of the cava is also muscular but, whereas in the dog and cat the hepatic veins open into the cava throughout its course through the liver, in the goat the veins opening into this part of it are few and small, and the main hepatic veins open into it as it is emerging from the liver and losing its muscular coat, and have, accordingly, no thickening of muscular coat near their orifices.

Quite recently Popper [1931] has published a preliminary survey of a very thorough investigation of the structure of the portal and hepatic veins in the dog, the cat and man, of which a fuller and illustrated account is promised. He finds that, in the dog, the portal vein and its branches are but poorly supplied with plain muscle, whereas the hepatic veins have much stronger coats of plain muscle, with the fibres mainly longitudinal, and arranged in columns. When these muscular columns are contracted, as in a liver fixed under the action of histamine, they cause projection of the intima into the lumen, so that the latter appears star-shaped in section, and may be almost obliterated. At the same time the lymphatics in the wall of the vein, collapsed in the uncontracted vein, are stretched open. In the cat Popper finds relatively little plain muscle in the hepatic veins, and no appearance suggesting such a restriction of lumen as is seen in the dog. The hepatic veins in the human liver resemble those of the cat rather than those of the dog; but appearances are seen in some livers suggesting the possibility of a throttle action where smaller open into larger veins. Though Popper emphasizes the correspondence of his anatomical findings with the obstruction to outflow from the dog's liver by histamine and shock poisons, his description does not account for the localization of the main actions of histamine and adrenaline, in what may be regarded as physiological doses, in the neighbourhood of the caval

openings of the main hepatic veins, as seen in our own experiments. These correspond rather with Elias and Feller's description of a throttle mechanism, with this location, in the human liver. While, therefore, it may be said that the physiological reactions peculiar to the dog's liver have, in general, an anatomical counterpart, the data available are not yet sufficient to warrant a final conclusion as to the extent to which they occur, if at all, in species not yet tested physiologically, including man. With regard to the nature of the muscular actions, which create or remove the obstruction to outflow near the openings of the main hepatic veins in the dog, we have no clear evidence; we do not know, for example, whether variations of tone in the rich muscular coat of the intra-hepatic cava itself contribute to the effects. It may be, further, that the physiological differences between species are not entirely due to differences in structure and contractility of the hepatic veins and their junctions with the cava. Possibly the network of capillaries or sinusoids in the lobules of the dog's liver is more sensitive to the pressure caused by even slight resistance to outflow, than that in the cat or the goat. The relative ease with which the dog's liver becomes "blood-logged" with relatively brief mechanical obstruction to outflow, as by accidental kinking of the vena cava, suggests such a difference.

Discussion.

There remains for discussion the physiological meaning of the mechanism of resistance to the outflow from the dog's liver, and its variation under chemical and nervous influences. The augmentor effect on this resistance of "shock poisons"—peptone, histamine, the specific antigen in an anaphylactic dog—has long been known. The gradual increase of the resistance during artificial perfusion of the dog's liver is probably due to an effect of the same kind. It is impossible, under the best conditions, to perform an artificial perfusion without some injury to the liver cells. Any such injury will lead to the liberation of "histamine-like" substances; histamine itself can be isolated from an alcoholic liver extract [Best, Dale, Dudley and Thorpe, 1927]. It is not unreasonable to suppose that during digestion traces of such substances, reaching the portal blood, will tend to raise the resistance to outflow, and thus cause the accumulation in the liver of a larger fraction of the total blood. Under such conditions a mechanism is needed by which a part of this floating reserve may be released into the general circulation at the call for muscular activity; and this is provided by the action of the sympathetic impulses and of adrenaline, which the experiments of Grab, Janssen and Rein

[1929 *b*] have shown to be effective with the liver in its natural relations. The whole mechanism, indeed, when thus considered, appears to be so well adapted to adjustment of the current blood volume to the body's needs, that failure to find it in other species is more surprising than its discovery in the dog.

Fuller discussion of this function of the liver as a variable reserve of blood is beyond the scope of this paper, but reference may be made to papers by Krogh [1912], by Jarisch and Ludwig [1927], and by Grab, Janssen and Rein [1929 *b*]. The point which we desire to emphasize, in this connection, is that evidence for such a function of the liver in the dog is not affected by failure to demonstrate it by other methods in other animals, such as the cat.

Summary.

1. A scheme for complete perfusion of the liver, with records of arterial and portal inflows, hepatic outflow and liver volume, is described.

2. In addition to vaso-motor controls of the inflow, the outflow from the dog's liver is controlled by a sluice mechanism, closed by histamine and opened by adrenaline or by sympathetic nerve impulses.

3. This sluice mechanism is located near the caval orifices of the main hepatic veins. Both adrenaline and histamine appear to produce a relatively weak constriction of deeper veins.

4. This variation of resistance to outflow is not observed in the liver of the cat, and is weak and inconstant in that of the goat.

5. The relation of these differences to the anatomy of the hepatic veins, and the physiological function of the mechanism seen in the dog's liver are discussed.

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THE INFLUENCE OF WORK ON THE CALORIGENIC ACTION OF THYROXINE.

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The active principle of the thyroid gland, isolated by Kendall in 1914, has been the subject of much investigation [Kendall, 1915 *a*, *b*, 1917; Harington, 1926; Harington and Barger, 1927; Lyon and Redhead, 1927; Lyon, 1927; Abderhalden and Hartmann, 1927]. Plummer and Boothby [Plummer, 1920, 1921, 1926. Boothby, 1920; Plummer and Boothby, 1921, 1924] showed that equal doses of thyroxine given intravenously produced strikingly constant results. Boothby and Baldes [1926] and Boothby, Sandiford, Sandiford and Baldes [1926] have shown that the "decay curve" (that is, the curve representing the return of the basal metabolic rate to its original level) after a dose of thyroxine is remarkably constant where the same myxœdematous patient is concerned, and even in different persons the variation is not great. They showed that this decay curve is exponential in type and that from 4 to 6 p.c. of the amount of thyroxine present in the body disappears daily as evidenced by decrease in the basal metabolic rate. Individuals therefore will be more or less sensitive to a given dose according to its rate of elimination or destruction, and the effect of 10 mg. injected intravenously into a human myxœdematous patient will not entirely disappear for several weeks. As emphasized by Plummer and Boothby the importance of this fact in the treatment of myxœdema is very great.

The remarkable constancy of the effect of thyroxine in the same person, and even in different persons, having been established, it became of interest to find out the causes of the relatively slight variations found,

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and to discover, if possible, factors which may produce more rapid disappearance of the thyroxine. Such a study was impracticable on the human being on account of the slow disappearance of the effect of administration of thyroxine and the impossibility of keeping subjects under constant conditions for such long periods. Abderhalden and Wertheimer [1926] obtained a greater effect on rats from the same dose of thyroxine when these animals were fed on a high protein diet than when they were given a low protein and high carbohydrate diet. Abelin, Knuchel and Spichtin [1930] have reported some very interesting experiments which indicate that a diet rich in vitamines decreases the harmful effect of large doses of thyroxine when administered to rats. Casein seems to have a somewhat similar effect [Abelin, 1930 *a*, *b*].

The experiments which form the basis of this paper were designed to find out, if possible, whether the rate of disappearance of thyroxine could be altered by muscular work. It was thought possible that the increase in heat production due to the work might increase the rate at which thyroxine was destroyed. For these studies the dog seemed the best type of animal to use, for the rate of the destruction of thyroxine is more rapid than in the human being and the original level of heat production is regained in from 1 to 2 weeks after the injection of 10 mg. intravenously. But, in the dog which has not undergone thyroidectomy, the possible effect of the thyroid gland itself must be borne in mind, and, on the other hand, in the thyroidectomized dog the influence of the tetany due to post-operative parathyroid insufficiency may be a complicating factor.

As part of our controls, we used the experiments recently reported by Wilhelmj and Boothby [1930], which show primarily that a single intravenous injection of thyroxine produces a remarkably constant calorigenic effect in the normal unthyroidectomized dog, fed on a standard diet, without attention being given to its iodine content. Secondly, they demonstrate that administration of an excess amount of iodine in the form of compound solution of iodine both before and during the thyroxine reaction, does not appreciably alter the effect, as can be seen in Fig. 1. On the other hand, the experiments of Boothby and Wilhelmj [1931] show that in the same dog after thyroidectomy the calorigenic response is materially greater, due in great part to a slower return to the prethyroxine line. Neither in the thyroidectomized, nor in the unthyroidectomized dog are the decay curves as even as those reported for human beings by Magnus-Levy [1904] and by Boothby, Sandiford, Sandiford and Slosse [1925]. However, the curves do fall off slowly and progressively, although irregularly, and bearing in mind the experimental

difficulties, are consistent with the exponential type of curve found in the human subject.

It seems likely, therefore, that the greater heat production from a given dose of thyroxine in a thyroidectomized animal is due to the slower rate of destruction or elimination of thyroxine at the lower levels of concentration that are present. In the dog with a normally functioning thyroid gland the average concentration would, of course, be much higher, since the thyroxine injected is added to that normally present. If the exponential character of the decay curve of thyroxine is assumed to hold for the two conditions, and the rapidity of its disappearance by destruction or

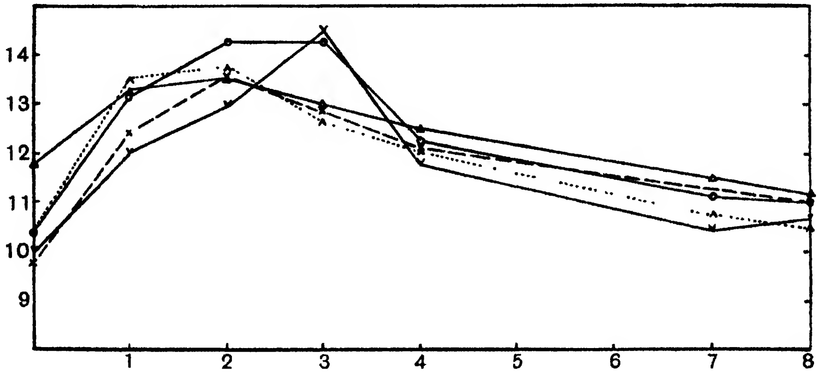

Fig. 1. Shows the close similarity in calorigenic response to equal doses of thyroxine given to a normal unthyroidectomized dog on five separate occasions. Before and during the periods covered by the dotted curves iodine was being administered to the dog, but caused no appreciable change in the response to thyroxine.

elimination is primarily dependent on the concentration in the body, then it follows that the excess heat production will be greater after thyroidectomy than before. The data of Boothby and Wilhelmj are in agreement with such an interpretation.

Methods.

The present series of experiments on the effect of work can be divided, for convenience of description, into three periods:

Period 1. During the first period no exercise was given; the effect on the metabolism of five successive doses of thyroxine was observed.

Period 2. During the second period the dog was made to walk on the treadmill nearly every afternoon, after the morning's respiratory metabolism tests. The after-effect of exercise at this time could not be a

complicating factor in the determination of the respiratory metabolism next morning. Under these conditions the effect on the metabolism of four successive doses of thyroxine was observed; with each dose the amount of work was progressively increased until the dog walked approximately 10 miles in the afternoon and at times was very tired.

Period 3. In the final period the exercise was discontinued; two doses of thyroxine were given and their effect on the metabolism studied.

In each case 10 mg. of thyroxine were injected into the internal saphenous vein.

The experiment was carried out on a normal unthyroidectomized dog, which had been trained for studies of respiratory metabolism, and which was thoroughly familiar with all the experimental procedures. Her initial weight was 6·7 kg. Her diet was constant throughout, and she was kept in a metabolism cage, so that the excreta could be collected and analysed. The analysis of the excreta could not, however, be begun until the latter part of period 1. The metabolic rate was obtained in a series of tests during the morning, and the single meal of the day was given at noon. During the period when exercise was given, the dog was placed on the treadmill after the noon meal, and was returned to her cage during the afternoon or evening, according to the amount of exercise required. The determinations of respiratory metabolism taken during the morning were, therefore, uninfluenced by the after-effect either of food or of exercise. The technique used in the determination of heat production was that described by Boothby and Sandiford [1920] and adapted for animals by Kitchen [1924]. From the volume of oxygen absorbed and the respiratory quotient (not corrected for protein metabolism), the total calories consumed per hour were calculated. For each respiratory determination, twelve or more individual tests of 10 min. each were made, with an interval of approximately 3 min. between the tests. The heat production of the dog, as given in the chart, was calculated on the basis of the average of the lowest consecutive series of tests lasting approximately an hour. However, the interpretation of the significance of the respiratory quotient has been based on all the tests made. The environmental temperature was kept constant, and the animal protected from disturbance by noise.

The total calorigenic response to a dose of thyroxine was obtained by integrating with a planimeter the curve representing the total calories produced per hour, for the first 7 days following the injection of thyroxine. The accuracy of the determination of the excess heat production following any experimental procedure depends more on the constancy and accuracy

of the base line than on any other single factor, because the area beneath the curve can be altered greatly by a very slight raising or lowering of the base line. As can be seen from the curve of total heat production in Fig. 2 the base level of heat production decreased rather suddenly in the latter part of the first period, and it was impossible to know with exactness just what base line should be used. The cause of this drop in heat production will be discussed later; the only point to be emphasized here is the fact that it did drop, which renders our estimate of the calorigenic effect of thyroxine in the experiments of period 1, especially the fourth experiment, somewhat indefinite. In order to avoid bias in the selection of the base line, in each experiment the determination, made on the morning before the injection of thyroxine, was used, in spite of the fact that sometimes, as in the first experiment, this was higher than the average of the preceding few days.

Diet. Throughout the entire experiment the dog was on a constant weighed diet of 156 g. of heart muscle and cracker meal. During the experiment this mixture was analysed thirty times, as each batch was made up, and was found to have an average nitrogen content of 2·93 g. With the exception of the batch made up for the first 3 days, the highest nitrogen value was 3·12 g. and the lowest 2·69 g. Its heat value was determined in the bomb calorimeter ten times, and averaged 512 calories, which, corrected for the urea value of its protein content, gave a food value of 482 calories. At the beginning of the experiment the basal metabolism averaged 228 calories for each day; therefore the diet was slightly more than twice the basal value, and was estimated to be sufficient to prevent loss of weight as a result of the administration of thyroxine. It proved to be adequate for period 1, as the weight at the beginning and end of the period was the same; when work was superimposed on the administration of thyroxine in period 2, the diet was insufficient to prevent a loss of 0·9 kg., while in period 3 the diet was sufficient to permit a regain in weight of 0·2 kg.

Fæces. The nitrogen in the fæces was determined, and with it the nitrogen in the hair shed into the cage, for a period of 71 days; since the 10-day averages were nearly constant (0·33, 0·25, 0·20, 0·26, 0·20, 0·22, 0·20 g.), further analyses seemed unnecessary, and we have used the average figure of 0·24 g. as the daily fæcal nitrogen throughout the experiment; this figure also includes the nitrogen in any regurgitated food.

Urine. Beginning with the latter part of period 1, the urine for 204 days was analysed for its total nitrogen by the Kjeldahl method. The

urea nitrogen, and the nitrogen of ammonia, amino acids, creatine and creatinine were determined by the Folin methods. The difference between the total nitrogen and that contained in the sum of the partition products gave the residual, undetermined nitrogen.

RESULTS.

The data obtained in this series of experiments are charted in Fig. 2.

Nitrogen balance. Immediately after the injection of a single dose of thyroxine there is an increased excretion of nitrogen, producing a temporary sharp negative nitrogen balance of 2 or 3 days' duration; after this the elimination of nitrogen in the urine decreases very rapidly, producing a compensating period of several days of positive nitrogen balance; finally, as is shown in the chart, there is a slow rise in the excretion of nitrogen, until the output equals the intake and nitrogen equilibrium is attained.

The total nitrogen in the urine for 204 days averaged 2·65 g., and, as the nitrogen of the fæces and hair collected from the cage averaged 0·24 g., the average daily excretion of nitrogen was 2·89 g., against an average intake of food nitrogen of 2·93 g. The elimination in urine and fæces during the 25 days of period 1 averaged 2·92 g.; for the 115 days of period 2, with exercise, the nitrogen of the excreta averaged 2·90 g.; and for the 64 days of period 3, without exercise, 2·87 g. Therefore, between December 5 and June 26 the dog was, within the limits of experimental error, in average nitrogen equilibrium, in spite of the fact that she received seven intravenous injections of 10 mg. of thyroxine; furthermore, the exercise given in period 2 did not disturb the nitrogen equilibrium of that period.

There is direct experimental evidence that, during the last 25 days of period 1, the dog was in nitrogen equilibrium. Indirect evidence shows that nitrogen equilibrium also existed throughout the entire period, since there was no net loss of weight, whereas, during period 2, nitrogen equilibrium was maintained in spite of a moderate loss of weight.

Reserve of deposit protein. Boothby, Sandiford, Sandiford and Slosse [1925] showed that the continuous administration of small daily doses of thyroxine to a subject on a constant nitrogen intake caused only a temporary increase in nitrogen elimination, during the period in which the heat production was being elevated. After the heat production had reached its maximum, it was maintained there by the continued administration of a constant daily dose of thyroxine; the nitrogen elimination

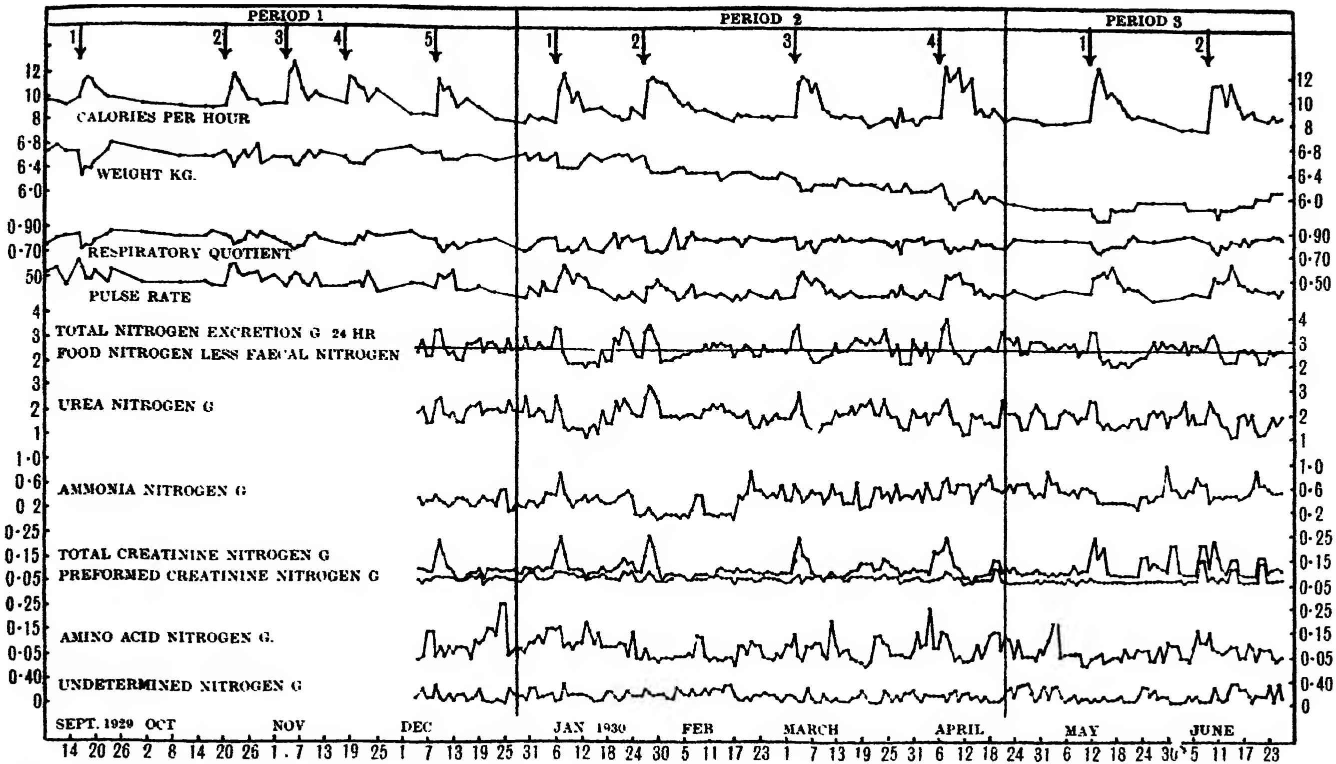

Fig. 2. Shows the effect of eleven intravenous injections of 10 mg. of thyroxine on the metabolism of a normal unthyroidectomized dog, over a period of 9 months. During period 2 the dog was given exercise on the treadmill, while in periods 1 and 3 it did no work. 1 ↓, 2 ↓, etc., indicate first, second, etc., dose of thyroxine, in the three periods respectively. The food nitrogen, from which the nitrogen contained in the fæces has been subtracted, is represented by a straight, horizontal line.

gradually decreased, until it was the same value as the nitrogen intake, thus re-establishing nitrogen equilibrium. As a result, the deposit protein in the body was reduced in amount, but remained constant at this new level as long as the thyroxine concentration was maintained at the increased level. Their experiments showed, therefore, that the concentration of thyroxine in the organism is a factor in determining the quantity of deposit protein. They were also able to show, by this method, that the œdema of myxœdema is an abnormal increase in the deposit or reserve protein. The present experiment shows that an amount of deposit protein, equivalent to that immediately lost as the result of the administration of a single dose of thyroxine, is regained, if a sufficient time interval elapses before such a dose is repeated.

Nitrogenous partition products. The data are presented in the chart, and it is evident that the temporary increase in elimination of nitrogen following injection of thyroxine is due largely to increased elimination of urea. The only other substance which is increased is the creatine nitrogen. Beumer and Iseke were the first to show that this is a common occurrence immediately after the administration of thyroid extract. Abelin [1930 *c*] has shown that the total creatinine content of both muscle and liver is decreased after the administration of thyroid substance. Boothby, Sandiford, Sandiford and Slosse [1925] showed, however, that the increase of creatine, like the increased excretion of nitrogen, is only a temporary phenomenon, when administration of thyroid is continued in daily therapeutic doses to myxœdematous patients. The creatinine nitrogen is very constant throughout and without consistent variation; we have rarely seen the creatinine nitrogen vary after administration of thyroid preparations more than can be accounted for by irregularity in the opening and closing of 24-hour collections of urine. The amino acid nitrogen fluctuates irregularly between 0·05 and 0·20 mg., a part of which irregularity is inherent in the method of determination. Similarly, the fluctuations of the undetermined nitrogen have no consistent trend, and the variations shown are also inherent in the method of estimation by difference. The variations in ammonia nitrogen bear no relation to the injection of thyroxine, and are largely dependent on the action of ammonia-forming bacteria, which can rarely be excluded from animal metabolism cages.

Weight. In period 1, following the first injection of thyroxine, there is a marked and rapid decrease in weight, followed by a gradual complete recovery. Following the other four injections of this period, a similar but less-marked reaction is observed. There is no net loss in weight throughout

the period, indicating that the diet was adequate to meet the caloric requirements of the dog, in spite of the intervals of increased metabolism due to the thyroxine.

In period 2 there is a similar sharp decrease in weight after each injection of thyroxine, but the recovery is less complete. In period 2 there is, in addition, a gradual loss of weight from 6·7 to 5·8 kg. This net loss of 0·9 kg. indicates that the diet was insufficient to meet the caloric requirements of the dog when exercise was superimposed on the increased metabolism caused by thyroxine. As the dog was in nitrogen equilibrium, the decrease in weight must have been due to the utilization of fat to meet the extra calories required on account of the exercise. As this was not pushed to the point of excess, the supply of fat was sufficient to prevent the utilization of protein for simple energy requirements. It can also be calculated that 0·9 kg. would furnish the approximate amount of energy needed for the exercise.

In period 3 there is a slight gain in weight, due to deposition of fat and not to increase in deposit protein, because, again, nitrogen balance is maintained for the period. This gain in weight shows that the energy requirements were more than sufficiently provided for throughout this period.

The basal heat production. The heat production has been expressed as total calories per hour, and the data presented in the upper curve in Fig. 2. As one studies the curve, it is obvious that after the exercise is started the calorigenic action of thyroxine is larger than at the beginning. However, in the first experiment, the heat production on the day the thyroxine was administered is definitely high; therefore, the estimated calorigenic action for that experiment is slightly low. The maximal increase in heat production is obtained, usually, on the second day after the administration of thyroxine, and is followed at first by a rapid, and then by a gradual, decrease in production of heat, and all but a very small part of the reaction is over by the end of the seventh day. The curve of decay is similar to the exponential type of curve found by Boothby in human beings with, however, greater irregularity. After the second and third experiments, we apparently did not wait long enough to allow the heat production to fall as low as it would, because, after the fourth experiment, there is a definite, and, at the time, an unexpected drop in the heat production, which is even greater after the fifth experiment. By the time the series of experiments, with exercise, of period 2 were started, the base line was between 1 and 1·5 calories (10 to 15 p.c.) lower than at the beginning of period 1. Although this decrease in heat production

interferes somewhat with the accurate estimation of the calorigenic action of thyroxine in period 1, the average error for the five experiments is probably not very great.

Since the dog was in nitrogen equilibrium and on a constant diet, with the same vitamin content. the sudden decrease of 15 p.c. in the rate of heat production on those days, during the latter part of period 1, which were not directly influenced by injected thyroxine must be accounted for by some other factor than a nutritional one, and it seems legitimate to consider the possibility that the repetition of injections of excess thyroxine at rather close intervals may cause a decrease in the activity of the thyroid gland in manufacturing thyroxine, because the normal demand of the tissues would presumably cease in the presence of an artificial excess. By repeating the administration of thyroxine at short intervals, just as the concentration from the preceding dose was reaching the normal level, there could. therefore, be partial or complete cessation of the activity of the thyroid gland. The normal stimulation of the thyroid gland would presumably not recur, until the concentration of thyroxine in the body dropped below the normal level. It is conceivable that after a considerable period of inactivity, due to artificial administration of thyroxine, there would be a material lag in the response of the thyroid gland to the normal stimulation of a decreased concentration. Because the basal metabolic rate of normal individuals is constant within narrow limits, Plummer deduced that there must be some type of mechanism controlling the output of the thyroid gland. Although our experiments do not prove that this mechanism can be influenced by administration of thyroxine, their evidence suggests that this may be so. Asher and his pupils [1926, 1928 *a*, *b*] have presented evidence which suggests that the sympathetic nervous system may be a part of this regulatory mechanism.

Calorigenic action of thyroxine. The increased heat production from 10 mg. of thyroxine, following the eleven administrations in the three periods, was as follows: during period 1, without exercise (except that which the dog could obtain in a small metabolism cage), the extra heat amounted, in the five experiments, to (1) 148, (2) 210, (3) 269, (4) 185, and (5) 288 calories, or an average of 220 calories.

During period 2, in which the dog was exercised on the treadmill in the afternoon up to a maximal amount, equivalent to a walk of 10 miles, the results of four experiments were (1) 428, (2) 450, (3) 387, and (4) 530 calories. or an average of 449 calories—nearly twice that of the first control period.

In period 3, again without work, the calorigenic action was, for exp. 1,

446 and, for exp. 2, 528 calories, or an average of 487 calories, which is even larger than during the work period.

These experiments show conclusively that the idea that muscular exercise, up to the equivalent of a walk of 10 miles daily, will cause a more rapid elimination or destruction of thyroxine, cannot be substantiated. The final control period, without exercise, shows that the increase found in the second period, when exercise was given, was not directly caused by the work. A large part of the increase in the calorigenic action in both periods 2 and 3 may be due to the probable decrease in the average concentration of thyroxine in the body, as evidenced by the lowered base line of heat production. This explanation could be deduced from the exponential character of the decay curve and the assumption that the rate of disappearance of thyroxine is dependent on its concentration. Indirectly, however, exercise *per se* might have an effect, which would exist for some time afterwards, through its influence in altering the ability of the muscles and other tissues to absorb and retain the thyroxine after injection. That is, the avidity of the tissues, especially the muscles, might be increased by moderate exercise following a confined life in a cage of more than a year, possibly by increasing the number of functioning capillaries. Consequently, after an intravenous injection, a greater proportion of the injected thyroxine would be rapidly absorbed from the blood stream and incorporated by the tissues as a functioning catalyst, before it could be destroyed or eliminated in the urine and bile. Kendall [1919] has shown that when a very large dose of thyroxine (200 mg.) is administered intravenously, 43 p.c. of the total amount of iodine contained in the thyroxine is excreted in the bile, and 13 p.c. in the urine, within 50 hours.

Pulse rate. Throughout the entire experiment, the pulse rate curve follows approximately the curve of heat production.

Respiratory quotient. Since the major effect of thyroxine, in altering the respiratory quotient, occurs during the first 4 days after each injection, the data have been separated into two divisions, as given in Table I.

Except for the 4 days immediately after the injection of thyroxine, the mean of the respiratory quotient determinations was 0·82 in period 1, without work, 0·81 in period 2, with work, and 0·83 in period 3, again without work. Although these variations are slight, they exceed significantly their probable errors (Table I). As the diet was the same for each period, the decrease in the average respiratory quotient of period 2 must indicate, for such a long period, either greater utilization of body fat, or less utilization of carbohydrate. The latter could not occur, for the energy requirement was increased by working on the treadmill.

TABLE I. Respiratory quotients.

	Observations on all days except the first 4 following injection of thyroxine			Observations on the first 4 days following injection of thyroxine		
Period	No. of observations	Mean respiratory quotient	Standard deviation	No. of observations	Mean respiratory quotient	Standard deviation
1	290	0·821 ± 0·002	0·004 ± 0·001	241	0·770 ± 0·002	0·035 ± 0·001
2	704	0·814 ± 0·001	0·040 ± 0·001	177	0·754 ± 0·002	0·030 ± 0·000
3	259	0·831 ± 0·001	0·029 ± 0·001	86	0·764 ± 0·002	0·026 ± 0·001

Although the heat production of this particular dog was not determined while she was actually exercising, it was determined on other dogs, and the extra heat produced was found to be about 20 to 25 calories for each hour under similar conditions. As the dog in the present series of experiments was made to walk 2, 3 or even more hours on most of the afternoons in period 2, it can be estimated roughly that the energy required for this work was, on the average, not far from 70 calories daily. During this period, the dog lost 0·9 kg. in 120 days, or an average of 75 g. daily. As she was in nitrogen equilibrium, this decrease in weight must be due to the utilization of body fat to meet the energy requirements of work, and the 75 g. would furnish 70 calories, or the amount equivalent to the estimated increased energy requirement.

Furthermore in period 1, as the dog was in weight equilibrium, the average respiratory quotient of 0·82 must have represented the average respiratory quotient of the food ingested. As the food eaten amounted to 482 calories, the diet contained, on the basis of a respiratory quotient of 0·82, 59·7 p.c. or 288 calories, derived from fat. In period 2, 70 more, or a total of 358 calories, must have come from fat, which would be 64·9 p.c. of the energy obtained from the food and body fat. The respiratory quotient for the utilization of 64·9 p.c. of fat would be 0·81, corresponding to that found experimentally.

The gain in weight during period 3 was 0·2 kg. or an average for the 60 days of 3·3 g. daily. As the dog was in nitrogen equilibrium, the gain must be due to fat and would represent about 31 calories daily. Calculating, as above, on the respiratory quotient 0·82 of period 1, representing equilibrium on 482 calories, the 288 calories derived from fat would be reduced to a net of 257 calories with a total energy expenditure of 451 calories. The fat then would represent 57 p.c. of the energy requirement and would be equivalent to a respiratory quotient of 0·83.

Over periods exceeding 2 months in duration there is, as one would anticipate, close agreement between the average respiratory quotients

found experimentally and those calculated from loss or gain in weight, on the assumption that the changes in weight, with the dog in nitrogen equilibrium, represented loss or gain of fat in periods 2 and 3 respectively.

In period 1 the immediate effect of thyroxine was to lower, for 4 to 5 days, the average respiratory quotient from 0·82 to 0·77; in period 2, from 0·81 to 0·75, and in period 3, from 0·83 to 0·76. The effect of a single, fairly large dose of thyroxine was to cause immediate lowering of the respiratory quotient, on the average by about 0·05. 0·06 and 0·07 for the first, second and third periods respectively; this persisted for about 4 days. The lowering effect of thyroxine on the respiratory quotient is apparently uninfluenced directly by the exercise given in period 2.

The calorigenic action of thyroxine increased the average basal heat production, in these 4 days of period 1, by 42 calories, of period 2, by 67 calories, and of period 3, by 70 calories daily. As has been seen above, the increased amount of fat consumed to meet this calorigenic action would, at the most, have lowered the respiratory quotient by 0·01. As the respiratory quotient was lowered by an amount six times as great as could be caused by the increased combustion of fat to meet the estimated calorigenic needs of the body as thus calculated, some other factor or factors must be concerned in the production of the temporary decrease. An experiment is now in progress in this laboratory which, it is hoped, will be sufficiently exact to permit deductions in regard to the cause of this discrepancy.

Summary.

1. The effect of eleven intravenous injections of 10 mg. of thyroxine on the metabolism of a dog over a period of 9 months, divided into three periods, was studied. In the first and third periods of 111 and 64 days the dog did no work. In the second period of 115 days it was exercised on a treadmill.

2. Throughout the experiment the dog was on a constant weighed diet, containing 2·93 g. nitrogen and 480 calories each day.

3. Average nitrogen equilibrium was maintained in all three periods. The urine nitrogen averaged 2·65 g., that in the fæces and hair shed 0·24 g., in all 2·89 g. daily.

4. Immediately after the injection of thyroxine there was a temporary negative nitrogen balance, followed by a compensatory positive balance after which equilibrium was restored.

5. During period 1 the respiratory quotient for all days except the

first 4 after each injection averaged 0·82; for period 2, 0·81, and for period 3, 0·83.

6. The dog lost weight in period 2. As she was in nitrogen equilibrium this loss must have been due to loss of fat, and the heat value of fat corresponding to the loss in weight was calculated to be equivalent to the energy of the work. It was also calculated that the combustion of this amount of fat would lower the R.Q. from 0·82 to 0·81, as found experimentally.

7. Similarly in period 3 the dog gained weight. The gain of weight was due to deposition of fat, since nitrogen equilibrium existed. The retention of this amount of fat would raise the R.Q. from 0·82 to 0·83, as in fact found.

8. During the first 4 days after injection of thyroxine the R.Q. was reduced to 0·77, 0·75 and 0·76 in the three periods respectively. This decrease was greater than could be accounted for by the amount of fat needed to meet the increased energy requirements.

9. The nitrogen excretion was increased immediately after injection of thyroxine, mainly owing to increased excretion of urea, and to a slight extent of creatine. The amounts of the other nitrogenous products did not vary significantly. Compensatory nitrogen retention followed for a few days, due to decreased excretion of urea.

10. Following exercise the decay curve of thyroxine decreased less rapidly than in the preliminary control period, and this effect continued throughout the final control period. The total calorigenic action of 10 mg. thyroxine averaged 220 calories in period 1, 449 calories in period 2 with exercise, and 487 calories in period 3. Exercise, therefore, instead of decreasing, increased the calorigenic action of thyroxine, and this increase persisted for many days after the exercise was stopped.

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SOME FŒTAL BLOOD-PRESSURE REACTIONS.

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THE low blood-pressure in the fœtus compared with that in the adult has long been recognized, but there appears to have been little investigation of the physiological aspect of the fœtal circulation since the work of Cohnstein and Zuntz [1884], who were mainly concerned with the morphology of the blood and the relative pressures and rates of flow in the umbilical vessels; they made the very interesting observation that venous pressure in the fœtus is much higher than in the adult and approximates to a value one-half of that in the fœtal arterial system. It would thus seem at first sight that the load on the fœtal right heart is relatively greater than in the adult, but, on the other hand, both auricles are filled from the venous side and the respiratory aid to venous return is lacking, so that the increased venous pressure is probably necessary to ensure adequate filling of both auricles. After birth, although anatomical changes take place fairly rapidly and bring the gross structure of the system into line with the adult conformation, the physiological changes are slower, and the adult blood-pressure level is not reached until after a period which is measured in weeks or months in some animals [Clark and Holling, 1931] and in years in the case of human beings [Sladkof, 1903, quoted by Feldman, 1920, p. 437]. In puppies, Clark and Holling have shown that the protein osmotic pressure of the serum shows changes with age which tend to compensate for the low blood-pressure in such functions as glomerular filtration in the kidney and perhaps lymph formation.

The experiments described below have been done on cats, but one experiment on a fœtal dog indicates that the changes described are similar in the two species. Pregnant animals as near full term as possible were used, but it was almost impossible to decide with accuracy before an experiment the actual duration of pregnancy; the stage was judged roughly by the size of the fœtal heads determined by palpation. The animals were anæsthetized with chloralose, the abdomen opened in the

midline and one fœtus delivered through an incision in the uterus and wrapped in cotton-wool, leaving the umbilical cord intact. The fœtal blood-pressure was recorded by a small mercury manometer attached to a specially made cannula which was inserted into the carotid artery; the maternal pressure was also recorded. It is necessary to exercise great care in putting the cannula into the diminutive fœtal carotid, because

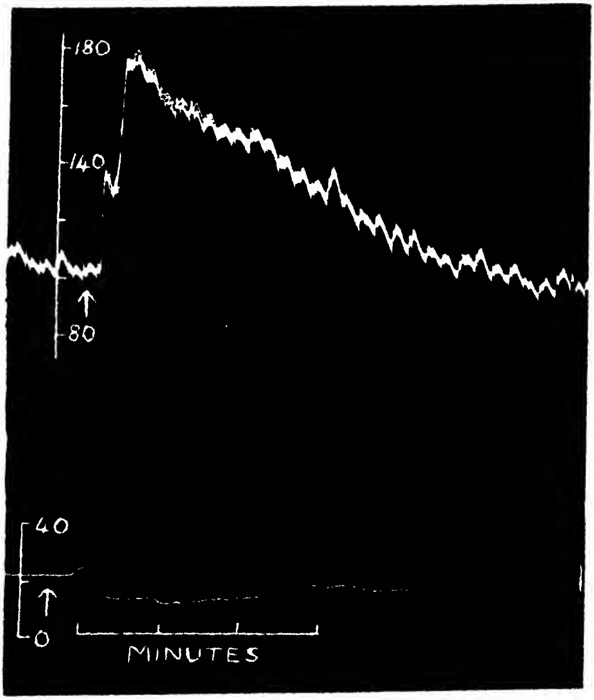

Fig. 1. Pregnant cat. Vagi cut. Upper record, maternal blood-pressure. Lower record, fœtal blood-pressure. 0·02 mg. adrenaline intravenously in mother at arrow.

this vessel is very friable, and the coats easily separate into two layers the inner of which tears and retracts, thus preventing free communication between the artery and the cannula. The fœtal pressure was found to vary in different experiments between 25 mm. Hg and 30 mm. Hg. It must be recognized, however, that this is the pressure in the fœtus outside the uterus; the fœtus *in utero* will probably have a higher pressure due to the uniform compression exerted by the uterine tone through the amniotic fluid. This will be the case more especially in the stages of pregnancy before the fœtal vaso-motor control is fully developed.

In the first experiment there were no spontaneous uterine contractions, and both maternal and fœtal blood-pressures were undisturbed by abnormal fluctuations. An attempt was made to determine the effect on fœtal blood-pressure of intravenous injections of various substances into the mother; it was found that adrenaline, pitressin and histamine, substances which caused different and easily distinguishable changes in the maternal circulation, all resulted in a fall of fœtal blood-pressure. Search-

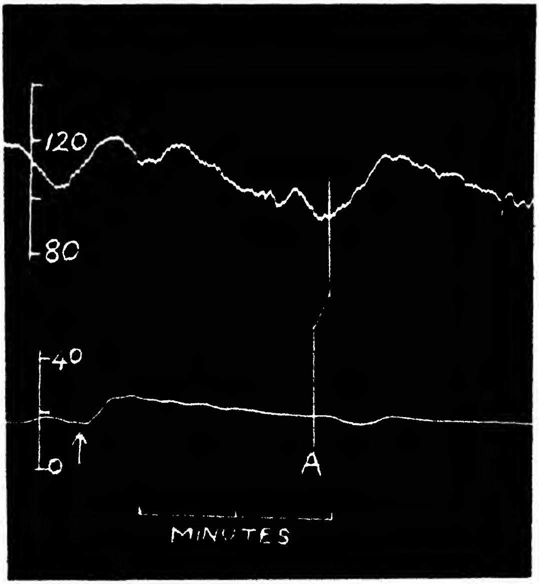

Fig. 2. Pregnant cat. Vagi not cut. Fœtal vagi cut. Upper record, maternal blood-pressure. Lower record, fœtal blood-pressure. 0·0002 mg. adrenaline intravenously in fœtus at arrow. *A*, marks onset of uterine contraction.

ing for an explanation of this, it was evident that one thing these substances had in common was that they all produced contraction of the pregnant cat's uterus, the pitressin effect being probably due to small traces of oxytocin; it is significant that the fall in fœtal pressure with pitressin was less than with adrenaline or histamine, although the resulting change in maternal pressure was of the same order. Fig. 1 shows the action of adrenaline. There can be little question of the fall in fœtal pressure being due to the passage through the placenta of a trace of adrenaline and causing vaso-dilation, for Fig. 2 shows that a minute amount of adrenaline injected intravenously into the fœtus is followed by

a rise in blood-pressure similar to but of relatively longer duration than that seen in the adult.

In subsequent experiments the uterus always showed spontaneous contractions, and in Fig. 3 it is seen that these were reflected in the maternal pressure curve by a rise which was maximum at the height of the contraction; the uterine contractions in this case were recorded by means of pulley and bell-crank lever. The rise of pressure occurred whether the vagi were cut or not in cats, but in one dog the rise was not seen with

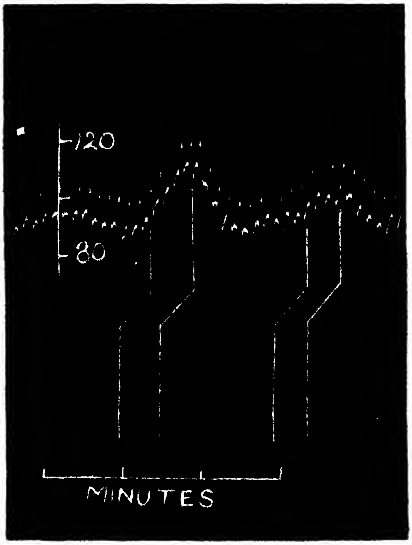

Fig. 3. Pregnant cat. Vagi cut. Upper record, blood-pressure. Lower record, spontaneous uterine contractions. Vertical lines mark simultaneous points.

intact vagi. Whether the increase in pressure was due simply to the squeezing of blood from the uterine vessels or to a general sympathetic stimulation could not be determined, as any attempt at clamping the uterine vessels, or, in fact, any interference with the uterus, brought on a rise in blood-pressure which was usually greater than that due to spontaneous contractions.

In the fœtus, accompanying each contraction of the uterus the most obvious effect on blood-pressure was a fall which lasted throughout the whole contraction, but this fall was preceded by a slight and transient rise (Fig. 4). The effect in fact is identical with that seen as a result of adrenaline in Fig. 1. The initial transient increase in pressure was pre-

sumably due to squeezing of blood from placenta to fœtus, but the explanation of the fall was not so evident. It could not be due to nervous reflexes arising in the placenta, for there is no nervous tissue in this structure [Schmitt, 1925]. If the fall was due in some way to obstruction of the fœtal circulation through the placenta, clamping the umbilical vessels ought to bring about a similar result, and, as Fig. 5 shows, this was the case; a contraction of the uterus occurred while the vessels were closed

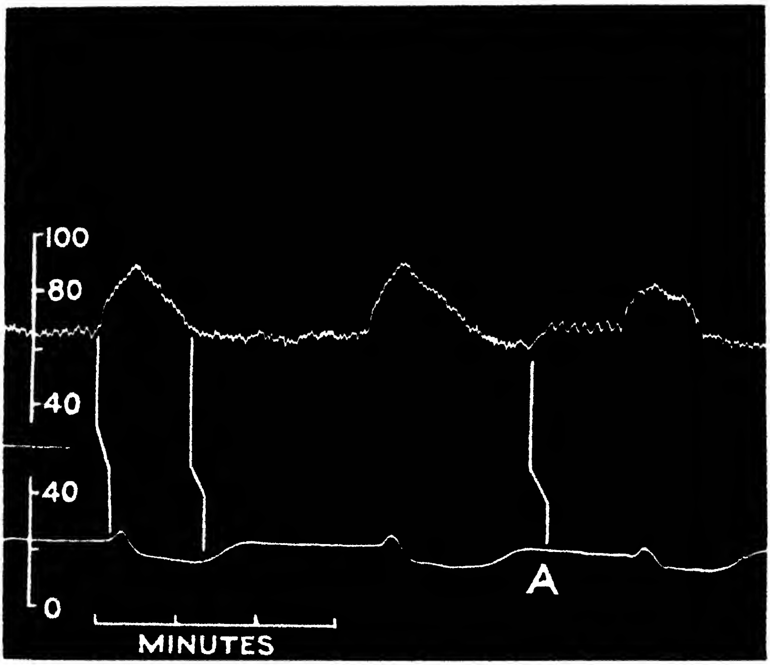

Fig. 4. Pregnant cat. Vagi cut. Upper record, maternal blood-pressure. Lower record, fœtal blood-pressure. Vertical lines mark simultaneous points. At *A* maternal trachea clamped.

without any corresponding change in fœtal pressure. The degree of abruptness with which the decrease in pressure took place suggested a fœtal depressor reflex as a possible cause, but section of both vagi (Fig. 5 *A*, *B*) failed to influence materially the pressure level, which was already slowly rising. The clamp was removed in the course of a uterine contraction (Fig. 5 *Y*), with the result that the pressure rose well above the normal level, to fall again as the uterus relaxed. A second contraction resulted in a change in fœtal pressure similar to that seen in the mother, but the fœtus died a

few minutes later. At first sight this altered fœtal response to uterine contraction after section of the vagi seemed to indicate that these nerves might play an active part in the production of the normal reaction and suggested that the fall might be a coordinated attempt to compensate for the rise in fœtal pressure which must otherwise occur mechanically *in utero* due to compression of the body of the fœtus during a contraction.

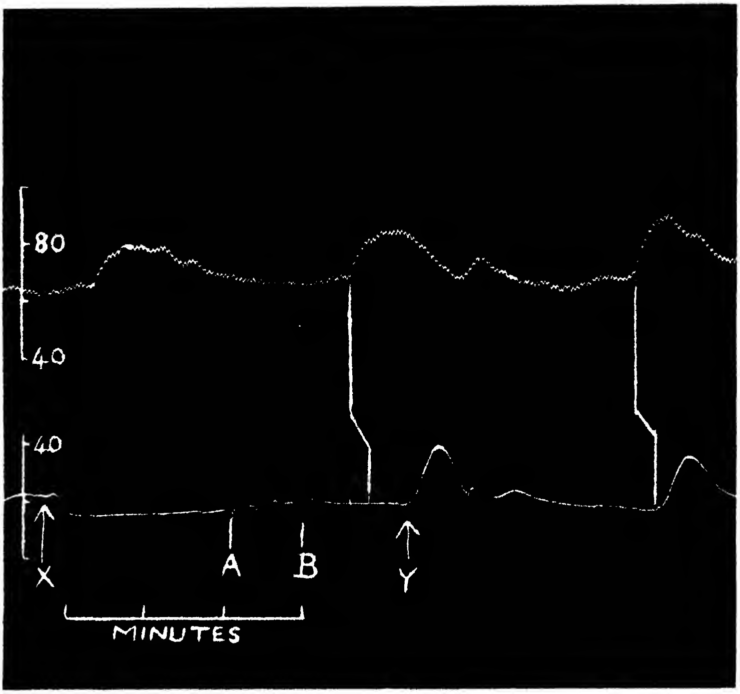

Fig. 5. Pregnant cat. Vagi cut. Upper record, maternal blood-pressure. Lower record, fœtal blood-pressure. At X umbilical vessels clamped; released at Y. Left fœtal vagus cut at A, right at B.

Subsequent experiments did not confirm this view however, because it was found that the type of change shown in Fig. 4 could occur even after the vagi were cut early in an experiment, and also after the left carotid was clamped and the right carotid sinus reflexes rendered ineffective because of the presence of a cannula in the vessel (Fig. 6). It was evident therefore that the fall in fœtal pressure during a uterine contraction was not due to the accepted depressor reflexes. The apparent evidence to the contrary in Fig. 5 is inadmissible because the fœtus was moribund and both blood-

pressure and heart rate were definitely below normal in the interval between contractions of the uterus.

The question then arose whether the fall in fœtal pressure was cardiac in origin or due to vaso-dilation. In every case the fall was accompanied by cardiac slowing, and in Fig. 1 the fœtal heart rate fell to 48 per min.; this cardiac slowing during a uterine contraction was first observed in the human fœtus by Legumeau in 1822 [Feldman, 1920]. Close examina-

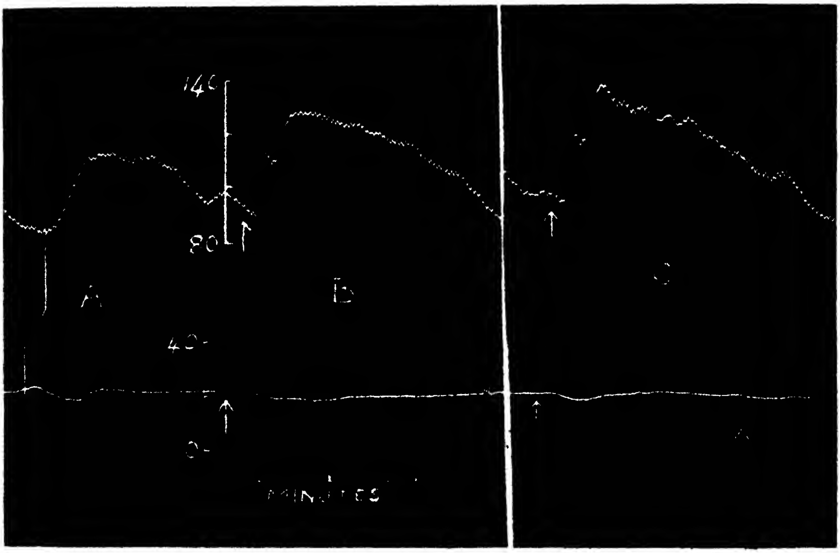

Fig. 6. Pregnant cat. Vagi not cut. Fœtal vagi cut. Upper record, maternal blood-pressure. Lower record, fœtal blood-pressure. *A*, spontaneous uterine contraction. *B*, 0·005 mg. adrenaline intravenously in mother. *C*, 0·005 mg. adrenaline intravenously in mother after clamping left fœtal carotid (arterial cannula in right).

tion of the records obtained, however, showed that reduction of the heart rate only occurs after the pressure has fallen and therefore cannot be the cause of the fall. It will be observed too that section of the vagi in Fig. 5 did not result in acceleration, in fact, the rate became slower, although this was probably not a result of the nerve section, as the heart was becoming progressively slower from the point at which the fœtal pressure was lowest. Moreover, there is evidence that the inhibitory effect of the vagus on the heart is either absent in the fœtus [Soltmann, 1877, quoted by Feldman, 1920] or feebly developed [Buglia, 1926; Kellog, 1927].

The high venous pressure in the fœtus observed by Cohnstein and Zuntz [1884] and the probable necessity for this in the fœtal circulation has already been referred to. Any obstruction of the placental circulation will immediately reduce this venous pressure; in the case of a uterine contraction the reduced venous return will be preceded by a transient increase due to the squeezing of blood from the placenta. The fall in venous pressure will be reflected in a diminished cardiac output, but when the umbilical vessels are clamped, not only is the venous return to the heart reduced but the arterial system is also diminished by shutting out the placental circulation, and it is not at first sight clear why this latter change should not compensate for the former. In clamping the umbilical vessels, however, there is rendered stagnant the volume of blood in the hypogastric arteries and in the umbilical veins as far as the ductus venosus in the liver; the absence of a flow of blood in the umbilical vein will naturally reduce the *vis a tergo* which normally plays a part in assisting the venous return through the liver, thus adding further to stagnation in that organ. It will be evident that all these factors tend to produce diminished venous return and so a fall in arterial pressure. During the time that the circulation in the umbilical vessels is obstructed, moreover, the fœtus must be suffering from partial asphyxia, and this is probably the cause of the cardiac slowing regularly seen, for Roaf and Sherrington [1910] and Lewis and Mathison [1910] have observed the onset of heart block in experimental animals within a few minutes of the cessation of ventilation, and both sets of workers agree that the phenomenon is not due to vagus activity. The oxygen content of fœtal blood is considerably less than that in the mother, while the carbon dioxide content is greater [Cohnstein and Zuntz, 1884; Huggett, 1927]. Some attempt was made to determine the effect of asphyxia by clamping the maternal trachea, but in this case it would be expected that the fœtal circulatory effect would be slower in onset than when the placental circulation is stopped, for in the former case the volume of maternal blood is available for gaseous exchange while in the latter there is no reservoir available other than the blood contained in the fœtus. Huggett [1927] reports that at a stage when the fœtal heart is distressed by asphyxia of the mother there is still a higher tension of oxygen and a lower tension of carbon dioxide in the maternal blood than in the fœtal. The result (Fig. 4 *A*) was not entirely unequivocal, although there is definite slowing of the heart from about 200 per min. to about 130 per min. and a tendency for the blood-pressure to fall; the intervention of uterine contractions prevented prolonged observation. This asphyxial slowing of the heart then will tend to keep

down the fœtal blood-pressure. An additional feature in these reactions is the relative lack of response of the fœtal vaso-motor centre to asphyxia: in Fig. 5 there is only a very slight rise in pressure despite nearly 5 min. asphyxia. But the sensitivity of the centre increases as the termination of pregnancy approaches, and Fig. 7 shows an experiment done on a fœtus within a few days of full term and about 10–14 days older than that from which Fig. 5 was obtained. In the case of the older fœtus contraction of the uterus caused a much less fall in blood-pressure than in the earlier one, and Fig. 7 shows that continued asphyxia caused a considerable rise of more rapid onset than in Fig. 5. In Fig. 7 the umbilical vessels were clamped between the two arrows, but on removal of the clamp the vessels remained contracted till the death of the fœtus about 20 min. later.

Fig. 7. Pregnant cat. Vagi not cut. Upper record, maternal blood-pressure. Lower record fœtal blood-pressure. Umbilical vessels clamped between arrows.

There is obviously a vast field for investigation in the physiological variations in fœtal circulation, and this paper is merely intended as a preliminary report pending the seasonal increase in experimental material of a more suitable nature than that afforded by the cat.

Summary and conclusions.

1. Simultaneous records of maternal and fœtal blood-pressure have been made in cats anæsthetized with chloralose.

2. Each uterine contraction causes changes in maternal and fœtal blood-pressure:

(*a*) In the mother the pressure rises to reach a maximum at the height of contraction; this change occurs in the cat whether the vagi are cut or not.

(*b*) In the fœtus a transient rise is followed by a well-defined fall which lasts throughout the contraction; these changes are also seen whether the fœtal vagi are cut or not and also after putting possible carotid sinus reflexes out of action. During the fall in pressure the fœtal heart is slowed.

3. It is suggested that the fall in fœtal blood-pressure described is due in the first place to a reduced venous return to the heart and is in part maintained by cardiac slowing resulting from partial asphyxia.

4. The response of the fœtal vaso-motor centre to asphyxia increases towards the end of pregnancy.

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612.26:612.448

THE EFFECT OF THYROID FEEDING ON THE OXYGEN CONSUMPTION OF THE HEART AND OF OTHER TISSUES.

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Dresel [1928] has reported that the oxygen consumption of hepatic tissue from animals previously treated with thyroxine is accelerated much more than the oxygen consumption of renal tissue from the same animals. The following experiments were carried out to determine the relative acceleration of oxygen consumption of various tissues in rats treated with thyroid extract, and to compare the elevation of metabolism of the entire organism with that of various parts of the body, such as the hind-quarters, the abdominal viscera, and the heart-lung preparation. Litter mates, on a similar diet without thyroid extract, were used as controls. The oxygen determinations and operative procedures were conducted in the same way in both groups.

Methods.

The spirometer and CO_2 absorption tube previously described [Dock, 1931] were used to measure the oxygen metabolism of intact rats, of the splanchnic region and of the hind-quarters. The difference in oxygen consumption by the rat before and after ligating the arterial supply was assumed to be the rate of consumption in the tissues isolated by the ligature.

For measurement of the oxygen consumption of the heart-lung preparation we used a spirometer made from a 10 c.c. "luer" syringe, graduated in 0·2 c.c. After inserting a tracheal cannula which was connected to the spirometer by a tube containing soda lime, and then opening the thorax, artificial respiration was maintained by connecting the outlet tube of a "Master's" artificial respiration apparatus with the upper chamber of the spirometer. With this device, and by varying the size of the opening between the upper chamber of the spirometer and the outside air, it was

possible to maintain any desired rate and amplitude of motion of the spirometer bell. The arteries and veins to the head and upper extremities were ligated, a clamp was placed on the inferior vena cava, and the aorta was ligated at about the level of the left carotid artery. The circulation was thus reduced to the coronary and pulmonary circuits. Blood was admitted into this circuit by releasing momentarily the clamp on the inferior vena cava until the aorta was full and pulsated strongly, and the heart was slightly larger than when the circulation was intact. After immersing this whole preparation in Locke's solution at 35–37° C., the rate of oxygen consumption was recorded in periods representing the time required for the absorption of 1 c.c. (of oxygen). Two to six periods were measured in each experiment, the average number was three periods in the first series of ten experimental and ten controls, and four periods in the second series. During each period the heart rate was recorded with the electrocardiograph from electrodes immersed in the water bath. At the end of the last period the heart was weighed. In the first series of experiments it was tied off quickly and weighed with the contained blood to determine the heart volume corresponding to the last measurement of oxygen consumption.

The rats in the experimental and control groups were under identical conditions except that the former received 0·25 p.c. U.S.P. thyroid mixed into the stock diet for 5–7 days before the determinations of oxygen consumption. Small rats eat more food, and on a diet containing thyroid have a larger increase in metabolism than larger ones. The rate of oxygen consumption per unit of surface area (based on the formula 11·34 × (body weight)$^{2/3}$) was used to express the results of all measurements. In some preliminary observations it was found that the effects of thyroid feeding are more apparent in anæsthetized than in unanæsthetized rats at room temperature (19° C.). Thus, in six control rats the average O_2 consumption per 100 sq. cm. was 2·16 c.c. per min. before and 1·05 c.c. after anæsthesia, in the thyroid-fed group the average figure was 2·60 c.c. before and 1·79 after anæsthesia. Thus the basal metabolism was 70 p.c. greater, although the metabolism in unanæsthetized animals at room temperature was only 20 p.c. higher in the thyroid-fed group. This is similar to the effect of cold in concealing the specific dynamic action of protein in rats. Chloretone anæsthesia was routinely used except in ten experimental and ten control animals used to provide heart-lung preparations. In these tracheotomy was done under ether anæsthesia, the rats were decerebrated and artificial respiration started before opening the thorax.

Results.

The results of the measurements of rate of oxygen consumption of the splanchnic region (the tissues isolated by ligature of the hepatic, cœliac and superior mesenteric vessels) and of the hind-quarters (tissues isolated by ligating the abdominal aorta and inferior vena cava caudad to the renal vessels) are summarized in Table I. The increase in metabolism of these tissues is the same, within a few p.c. and well within the limits of experimental error, as that of the intact animal. There is no difference in the thyroid effect on resting muscle and on the large glandular structures such as the liver. It is obvious that the small rats eat more of the thyroid-containing diet and have a much larger increase in metabolism than the older rats used for the abdominal aorta ligations.

Table I. The oxygen requirement of isolated tissues of the rat estimated by comparing the oxygen consumption immediately before and after ligating the blood supply, in one series, of the splanchnic region, in the other, of the hind-quarters. The mean values for each group of animals are in heavy type, the maximal and minimal observed figures in light type. There were five thyroid-fed and five control animals in the splanchnic series, nine of each in the other series. Rates of oxygen consumption are related to the calculated area of body surface.

	Weight of rat g.	O_2 per min. per 100 sq. cm. Before ligation c.c. per min.	After ligation c.c. per min.	O_2 for isolated tissues per min. per 100 sq. cm. c.c. per min.	p.c. increase total metabolism	p.c. increase metabolism of isolated tissue
Splanchnic ligation:						
Thyroid group	**48·8**	**1·40** 1·33–1·48	**1·05** 0·98–1·13	**0·35**	+67	+75
Control group	**55·2**	**0·84** 0·79–0·94	**0·64** 0·59–0·73	**0·20**	—	—
Ligation of abdominal aorta:						
Thyroid group	**105**	**1·24** 1·15–1·41	**1·10** 0·98–1·19	**0·14**	+37	+40
Control group	**112**	**0·90** 0·85–0·98	**0·80** 0·73–0·85	**0·10**	—	—

About sixty rats were used in the studies of oxygen consumption by the heart-lung preparation. In many of the early attempts the heart rapidly failed, due apparently to our having admitted an inadequate amount of blood into the system. Some later observations were discarded because of the early appearance of pulmonary œdema, or the rapid fall in rate of oxygen consumption. In Table II are summarized the findings in forty satisfactory experiments, twenty on thyroid-fed and twenty on control rats. The first ten of each group were done on chloretone anæsthetized rats after determining the rate for the intact rat, the second series

of ten each were those in which ether anæsthesia and decerebration were used to avoid the cardiac depression following chloretone. No attempt was made to record heart volume or blood-pressure during the determinations of cardiac oxygen consumption. It was hoped that by using a simple experimental system and a fairly large number of animals the average heart volume would be about the same in the control and experimental groups. Actually, in the chloretone series, the average heart-volume/heart-weight ratio in the experimental group was 5 p.c. less than that of the controls, at the conclusion of the last period of observation.

TABLE II. The oxygen consumption of heart-lung preparations from normal and from thyroid-fed rats. The figures in heavy type are the mean of observations on groups of ten rats, the figures in light type are the maximal and minimal rates for individual rats in each group. In the two groups in which chloretone anæsthesia was used the total oxygen metabolism of the anæsthetized rat was measured, but this could not be done in those which were given ether in order to permit cutting the brain stem and then given artificial respiration.

	O_2 per min. per 100 sq. cm. c.c.	O_2 per min. for heart-lung c.c.	Weight of heart mg.	Rate of heart per min. beats	O_2 per g. of heart per beat c.mm.
Chloretone anæsthesia:					
Control group	**0·80** 0·71–0·93	**0·36**	**795**	**289**	**1·58** 1·12–2·14
Thyroid group	**1·07** 0·98–1·21	**0·49**	**942**	**327**	**1·60** 1·32–2·28
Increase in thyroid group	+34 p.c.	+36 p.c.	+18 p.c.	+13 p.c.	+2 p.c.
Ether anæsthesia:					
Control group	—	**0·45**	**705**	**331**	**1·95** 1·60–2·33
Thyroid group	—	**0·59**	**804**	**374**	**1·98** 1·82–2·34
Increase in thyroid group	—	+30 p.c.	+14 p.c.	+13 p.c.	+1 p.c.

The averages given in Table II are for the first period, that is the time required for the absorption of 1 c.c. O_2. The rate of oxygen consumption, in both groups of rats, was greater in those under ether anæsthesia than in those under chloretone, but the differences between controls and experimental groups, as regards heart weight, rate, and rate of oxygen consumption, were about the same in the two series. Both rate and weight of the heart were increased in the thyroid-fed rats, but the oxygen consumption, per gram of heart muscle per beat, was the same as in the controls. The increase in oxygen consumption per minute by the hearts of the thyroid-fed rats averaged 33 p.c., the increase in total oxygen consumption was 34 p.c. Table III shows the average rate of oxygen consumption, per gram per beat, in successive periods of observations. There

is obviously a progressive decrease in rate of oxygen consumption in such preparations, due in part to decrease in heart volume as blood accumulates in the lungs. This decrease is most rapid in the thyroid-fed rats and in those under chloretone anæsthesia.

TABLE III. The rate of decrease in O_2 consumption per gram of heart per beat in successive periods of observation. Average of ten rats in each group. The decrease in O_2 consumption is stated in p.c. of the rate for the first period for each group of rats.

	Decrease in O_2 second period p.c.	Decrease in O_2 third period p.c.	Decrease in O_2 fourth period p.c.
Thyroid fed, chloretone anæsthesia	12	21	—
Control, chloretone anæsthesia	6	17	—
Thyroid fed, ether anæsthesia	7	17	27
Control, ether anæsthesia	8	16	20

DISCUSSION.

Because of the poor muscular efficiency [Kommerell, 1931] and the occasional occurrence of heart failure in hyperthyroid patients, it might be anticipated that an active muscular organ, such as the heart, would be less efficient in animals treated with thyroid. However, in rat hearts under the conditions of our experiment, there was no evidence of such a change. Although the total oxygen consumption of the heart-lung preparation per minute was increased to the same extent as the total metabolism (35 p.c.), this difference could be explained entirely by the increase in rate and weight of the hearts of thyroid-treated animals. The oxygen consumption, per gram per beat, was the same as that of the controls. As the rate of beat, in the thyroid-treated group, was 13 p.c. faster than in the controls, the volumes of oxygen used per beat were not strictly comparable. Starling and Visscher [1927] found that the oxygen requirement per beat, at constant diastolic volume, decreased with increase in rate of beat. In dogs' hearts the oxygen used per beat decreased 0·3–0·5 p.c. for each 1 p.c. increase in rate. If similar changes occur in rats, the increase in rate of beat, in the thyroid-fed group, should have caused a decrease of 4–6 p.c. in the oxygen required per beat. Allowing for such an effect of increased rate, the observed oxygen consumption per gram of heart per beat is only 5–7 p.c. greater in the thyroid-treated rats. In view of the experimental error with this method of study, and the possibility that the metabolism of the rapidly beating heart of the rat is less altered by changes in rate than is the case with dogs, it appears probable that the efficiency of cardiac contraction in thyroid-fed rats is within the normal range.

This result is in disagreement with the work of Ambrus [1929] who reported an increase in the rate of glucose utilization of cats' hearts after thyroxin, and of McEachern and Andrus [1931], who found increased oxygen consumption by the isolated auricle of thyroid-fed guinea-pigs. We have no satisfactory explanation of this discrepancy, but the method of study and animal species were entirely different in the other experiments. However, our observation that the oxygen consumption of thyroid-treated rats' hearts is quite normal agrees with Kommerell's [1931] conclusions concerning muscular contraction in hyperthyroid patients: "Der biologische Prozess der Energiegewinnung bei Morbus Basedowii nicht gestört ist....Der muskuläre Wirkungsgrad ist bei Morbus Basedowii derselbe wie beim Normalen." He based these conclusions on careful study of respiratory metabolism during measured exercise, and the finding that although the energy requirement for movement of the limbs was greatly increased, the cost of each added kilogram-meter of work was the same in hyperthyroid patients as in a normal individual.

Summary.

The oxygen consumption of the splanchnic area and of the hind-quarters of rats fed thyroid extract for 5–7 days was increased to the same extent as that of the entire animal.

The oxygen consumption, per minute, of the heart-lung preparations from thyroid-fed rats was increased to the same extent as that of the entire animal.

The increase in the oxygen consumption of the heart can be accounted for entirely by the increased rate and weight of the heart of thyroid-fed rats; the oxygen consumption per gram of heart per beat is the same in the thyroid-fed rats with a + 35 p.c. oxygen metabolism as in the litter mate controls.

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612.843.53

REGIONAL VARIATIONS IN SENSITIVITY TO FLICKER.

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Introduction.

It is generally stated that intermittent images formed in peripheral parts of the retina require a higher rate of alternation to abolish flicker than those formed at the fovea. This view is unreservedly supported for white light by observations of Aubert [1865], Exner [1870], Bellarminow [1889], Sherrington [1904], Lohmann [1908], and Woog [1919], who employed various methods for the production of flickering images. In no case does very intense illumination appear to have been used, and the objects probably all subtended more than 2° at the observer's eye. Many of those who frequent cinemas are aware that flicker, under the conditions there obtaining, is more pronounced in indirect than in direct vision.

While Charpentier [1890] invariably obtained the same result with small objects, he could detect no difference between centre and periphery with objects whose images were much larger than the yellow spot. Moreover a second series of experiments by Exner [1886], using a much smaller object than in his earlier series, gave the same fusion frequency for centre and periphery with intense stimuli, although at lower frequencies of alternation flicker was coarser in indirect vision. With more feeble illumination, flicker was abolished for central vision at speeds insufficient to cause fusion in the periphery.

Rupp [1869], however, is reported by Exner [1870] (who contradicts him) to have found discs with alternate black and white sectors, seen by reflected light, to cease flickering in indirect vision at slower speeds of rotation than when viewed directly. Braunstein [1903] too has reported that the centre of the retina is more sensitive to flicker than the periphery. Dow [1910] was only able to confirm the usual finding with weak illumination; at high illuminations he states that fine flicker may persist at the

fixation point when all trace of flicker is absent elsewhere in the field of vision[1].

Bellarminow [1889] found the rule to hold for coloured stimuli of moderate intensity. For red, yellow, and green, but not for violet, conditions were reversed at high intensities, *i.e.* the periphery became less sensitive to flicker than the fovea. Braunstein [1903] obtained similar results, except that the rule was also reversed for blue at high intensities. On a field subtending 4·5° Polimanti [1889] alternated spectral colours with white light. For short wave-lengths higher frequencies of alternation were always required to abolish flicker at 15° eccentricity than with central vision. For light of longer wave-length, however, the difference was much less marked, and beyond about 650 $\mu\mu$ conditions were sometimes reversed. Using monochromatic spectral colours, Allen [1909] found regions of the retina 10° and 20° from the fovea more sensitive than the centre to flickering lights of all wave-lengths. Dow [1910] reports the centre more sensitive to red flicker, and the periphery to green. Ives [1912] confirms this finding as regards red and blue of low intensities, but finds no difference between centre and periphery for very intense blue and red stimuli. According to Hardy [1920], who used a test object subtending 3·36°, the fovea is more sensitive than the periphery to red and yellow, while there is little to choose between different regions of the retina for blue-violet.

Conducted with improved technique and white light, recent experiments of Lythgoe and Tansley [1929] and of Granit and Harper [1930] provide further evidence. The former (pp. 29, 61) used a square object subtending 1° at the eye and determined the critical frequency for extinction of flicker both with central fixation and with seven peripheral fixation points ranging as far as 105° from the object. The luminosity of the object, at speeds sufficient to abolish flicker, was 93·6 metre-candles. With the dark-adapted eye and with the remainder of the field of vision in complete darkness, the critical frequency was highest for the fovea and progressively fell on passing out to the periphery. When the surrounding field was almost as bright as the object, the readings at 10° were higher than those at the fovea. Further out the values again fell, but the foveal figure was generally not reached until a fixation point about 70° from the object was used.

Granit and Harper [1930, Fig. 2] find that small objects (0·5–1·5°) give a lower fusion frequency in the periphery than in the centre, whereas large objects give a lower value in the centre than in the periphery. The brighter the object, the smaller must it be in order to give identical values in the two regions. At first sight these results seem in conflict with Charpentier's. Perhaps we may suppose that the latter's small objects

[1] Statements that have recently been made implying that Marbe [1896], Haycraft [1897], and (under certain intensities of illumination) Sherrington [1897] also observed higher fusion frequencies in centrally fixated objects than in the same objects when the images fell on peripheral regions of retina appear to be based on a misapprehension of the purpose and conduct of their experiments. They were concerned only with central vision.

subtended a visual angle of at least 2°, and that his large objects were very much larger than any used by Granit and Harper.

From the somewhat confusing literature, we are probably justified in concluding that flicker is more marked in the periphery than in the centre of the field of vision with moderate intensities of illumination and objects of moderate size, but that the contrary may often be found with small objects (subtending less than 1° or 2°) and with intense stimuli, and is especially apt to occur with coloured light of long wave-length.

In the hope of further elucidating the factors concerned in regional variations in the critical frequency for extinction of flicker, we decided to make series of measurements with a very small object at varying angular distances in a horizontal and vertical direction from the fixation point. Mixed white light at a range of intensities of moderate illumination has been used throughout. By the use of a small object, the complicating effects of areal summation, which is so marked a property of the peripheral regions of the retina [Kleitman and Piéron, 1929; Granit and Harper, 1930], are reduced to a minimum. The circular test patch actually employed subtended a visual angle of only 12′, thus giving a retinal image of diameter 54μ and area 2300 sq. μ.

From observations on the limits of visual discrimination, Helmholtz [1896] calculated that there must be 13,466 separate visual units per sq. mm. in the fovea. By a similar method Wertheim [1887] had previously obtained the figure 14,000. Salzer [1880] actually enumerated about 135 cones per 0·01 sq. mm. in this region of the retina in three still-born infants. If this is so, the image of our test patch was formed on approximately 30 foveal cones. But although these figures appear to have been widely accepted by subsequent writers, they imply a much sparser distribution than might be expected of elements whose diameter is about 3 μ. We have therefore examined microphotographs of tangential sections of the area centralis of the human retina. Andersen and Weymouth [1923, Fig. 12] reproduce one from Fritsch [1908], and another has been published by Fincham [1925, Fig. 7]. Both show between 120,000 and 130,000 cones per sq. mm. In the periphery of the retina Fincham's photograph (Fig. 4) shows about 90,000 rods and cones per sq. mm. A microphotograph by Heine [1900] shows more than 80,000 foveal cones per sq. mm. in a monkey. Some shrinkage may have occurred in the making of these preparations, but it is evident that Helmholtz' estimate was almost 10 times too small, and that in our experiments something approaching 300 receptor elements are involved.

Except in the centre of the retina, however, the number of afferent

paths by which impulses are conducted to the brain is probably much smaller than the number of receptors stimulated. 3·2 mm. (11° 40′) from the fovea Chievitz [1889] found the ratio of rod and cone nuclei to ganglion cells 10·75 : 1, 4·6 mm. (16° 30′) from the fovea 42·00 : 1, and 6 mm. (21°) from the fovea 80·00 : 1. The figures of Mureddu [1930], expressed in a different and, for our purpose, less helpful way, are not obviously inconsistent with those of Chievitz.

Method.

The apparatus employed is shown diagrammatically in Fig. 1. A vertical sheet of opal glass was illuminated from behind by an electric

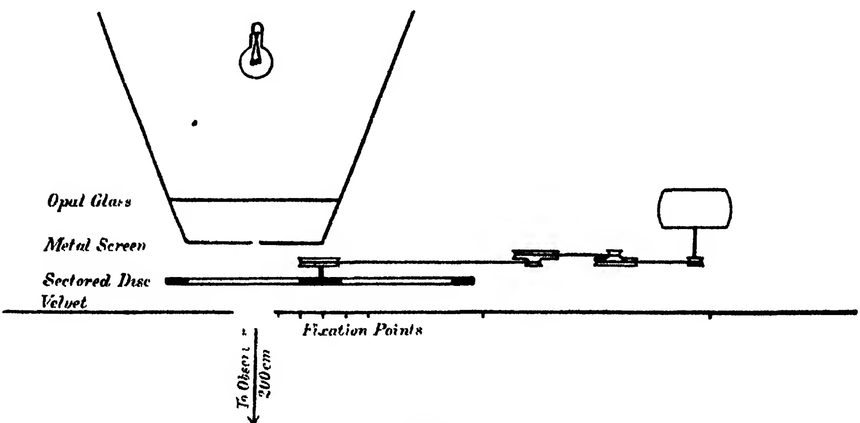

Fig. 1.

lamp. The intensity of the illumination was conveniently varied by employing lamps of different candle-power and by varying their distance from the glass. About 5 cm. in front of the glass was an opaque metal screen painted black and pierced by a hole 7 mm. in diameter. The small patch of white glass seen through this hole constituted the light stimulus to the observer's eyes. The hole was viewed from a distance of 2 m. and thus subtended only 12′ of arc. A large curtain of black velvet measuring 184 cm. horizontally by 90 cm. vertically was hung 13 cm. in front of the pierced metal screen. The bright object was observed through a round hole cut in this curtain 56 cm. from its left edge. Under the conditions obtaining during an experiment, the edge of the hole in the velvet was invisible. Uninterrupted blackness extended from the curtain to the test object.

A rotating black circular disc, with alternate equal sectors cut away, was

placed between the metal screen and the curtain. It was driven through reduction gears by an electric motor. The speed was controlled by the observer by means of a sliding resistance in series with the motor. Two discs were used: one with 18 open sectors, and another with 10.

To furnish fixation points for observations with peripheral parts of the retina, squares of white paper, measuring 3 mm., were pinned on to the velvet curtain. One series of these, six in number, was placed to the right of the test patch at distances subtending between 1° and 20° to the observer. Another was arranged vertically above the test patch to give displacements of 1° to 10°. They were feebly illuminated by a 5 c.p. lamp 430 cm. distant from the hole in the curtain, and behind and to the right of the observer. The observer's head was shaded from the direct rays of this lamp.

The experiments were performed in a room with black walls and ceiling. The only illumination was the lamp just described and some scattered light from the 6·6 c.p. or 46 c.p. source behind the curtain. Before each day's observations were begun, 20–30 min. were spent in becoming adapted to the low illumination. The observer was seated comfortably with his chin resting on a support. While gazing steadily with both eyes at one of the fixation points, he accelerated the motor until the flickering quality of the bright object gave way to a continuous sensation. When the speed at which flicker was just abolished had thus been found, the experimenter timed with a stop-watch 25 revolutions of the 18-sector disc or 50 revolutions of the 10-sector disc to the nearest tenth of a second. For this purpose the black posterior surface of each disc carried a patch of white paint on a short segment of its edge. Two stop-watch readings were taken, and a third in case of serious disagreement. The difference, however, did not as a rule exceed 0·2 sec. The same procedure was followed for other fixation points until the series was complete. Observer and experimenter then changed places.

All observations were made without knowledge of results, and the order in which they were made was varied from day to day. At least one of the earlier observations was repeated at the end of each series to check the reliability of the values obtained. The experimenter frequently also asked for repetitions of several determinations when irregularities appeared to be present. The first determination of a series was found in particular to be often unreliable. It has therefore been omitted in some of the records published below. With that exception, no readings have been concealed.

Evidence of the suitability of our method for the purpose in view is

afforded by the regularity of curves plotted from the results. At the same time, we do not wish to minimize its difficulty, nor do we claim a high degree of accuracy for any single figure. It is impossible to obtain a sharp end-point with so small an object. The long period of regard necessary makes fatigue inevitable, while with eccentric fixation, especially in the vertical meridian, spontaneous disappearances and reappearances of the object introduced a distracting element. Both obstacles were in some degree overcome by shifting the gaze or closing the eyes at intervals during the observations.

In some control experiments the fusion frequency with the 18-sector disc was compared with that with the 10-sector disc under otherwise identical conditions. The former, to our surprise, gave slightly, but definitely, higher rates than the latter. The finding, however, is in accordance with observations of Grünbaum [1897] and others, since the ratios of sector breadth to diameter of object in the two cases were approximately 2·5 : 1 and 4·5 : 1 respectively. For no single series was more than one of the discs employed.

The illumination of the object is expressed in arbitrary units based on the candle-power of the source and the distance of the source from the opal glass. If the glass could be assumed neither to reflect nor to absorb any of the light incident upon it, this unit would be equivalent to 26·3 metre-candles. It was found by measurement, however, that less than one-sixth of the incident light was transmitted, so that our unit is not in effect more than 4·4 metre-candles or 0·44 millilambert. When flicker is abolished, the apparent intensity is, of course, half that of the bright phase alone. Some of the higher illuminations may seem unduly intense, but it must be remembered that the subjective brightness is very greatly reduced by the smallness of the object.

Results.

1. *Object surrounded by black.*

Of 40 series obtained in the way described above, with intensities of illumination varying from 2·8 to 552 arbitrary units, all have shown a higher critical frequency for extinction of flicker in central than in peripheral vision.

When frequencies are plotted as ordinates on a graph relating fusion frequency with degree of eccentricity of the fixation point, the curve falls steeply from the value for foveal vision to that representing images 2° or 3° out towards the periphery. The changes occurring still further out are

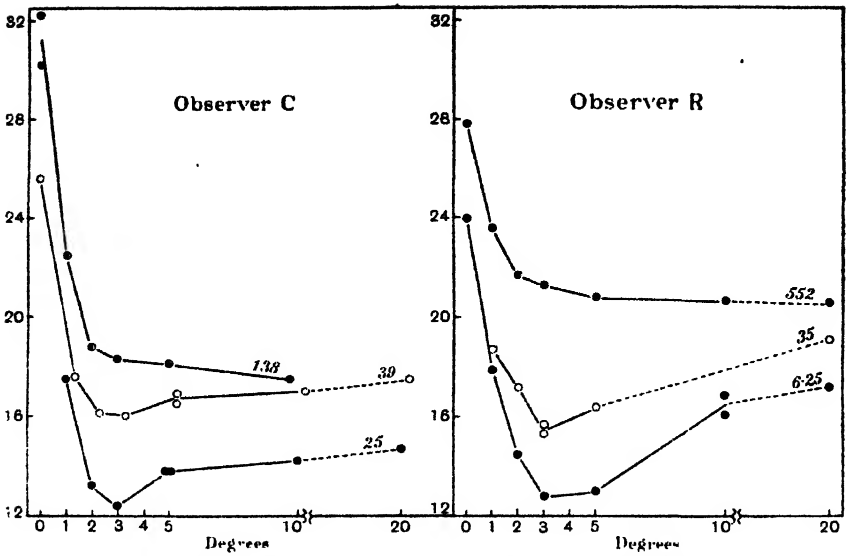

Fig. 2. Three series with each observer. All the results are plotted which were obtained in the single experiments for which each curve is drawn. Ordinates: fusion frequencies in flashes per second. Abscissæ: angular distances of fixation points from the object. The numbers above each curve express the brightness of the object (during its bright phase) in arbitrary units.

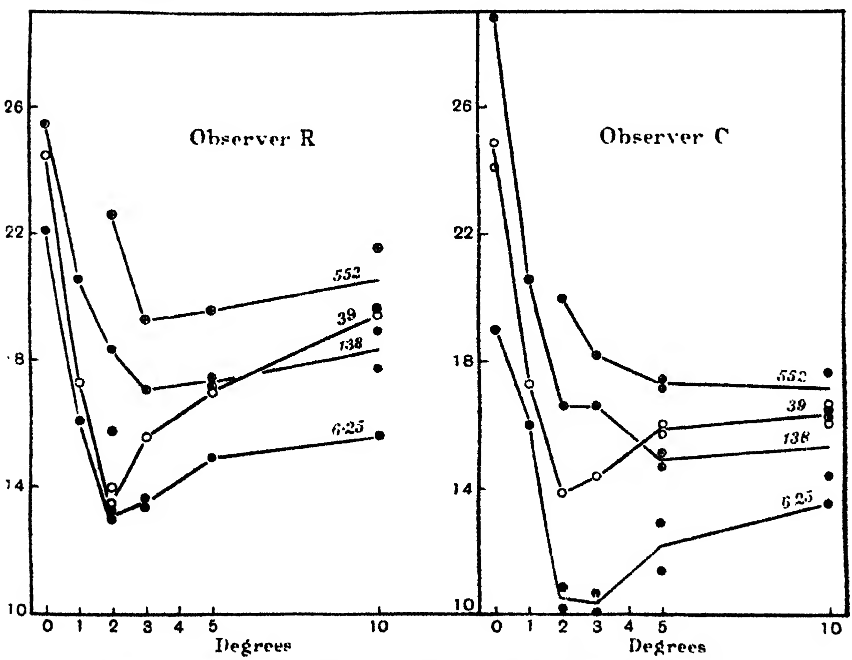

Fig. 3. As Fig. 2, except that the fixation points are vertically above, instead of horizontally to the right of, the object.

much less striking, but almost always there has been some increase in frequency on passing to more peripheral regions. Fig. 2 summarizes the results of several experiments in which horizontal displacements have been studied, while in Fig. 3 are shown similar curves obtained with the vertical series of fixation points. (No systematic comparison has been attempted of the vertical and the horizontal meridian. So far as our observations go, sometimes points on one and sometimes corresponding points on the other have given the higher readings.)

In such long experiments as these no doubt local adaptation and fatigue are liable to influence the results. The main features of the curve have therefore been checked and confirmed in experiments designed to

Fusion frequency in flashes per second for horizontal fixation points to right of object.

Observer *R*				Observer *C*			
1°	3°	20°	Notes	1°	3°	20°	Notes
—	16·0	—		—	11·5	—	
—	—	17·2	3° not flickering	—	—	14·1	1° and 2° only flickering
—	15.4	—	5°, 10°, and 20° flickering	—	11·2	—	
				17·5	—	—	No flicker elsewhere
19·9	—	—		—	—	14·0	
—	—	19·2	1° only flickering	—	11·5	—	1°, 2°, 5°, 10°, and 20° flickering
—	15·7	—					
—	—	17·8	1° only flickering				

Fusion frequency for vertical fixation points above object.

Observer *R*				Observer *C*			
1°	3°	10°	Notes	1°	3°	10°	Notes
17·7	—	—		—	13·1	—	1°, 5°, and 10° flickering
—	—	16·6	1° flickering; 3° not	19·2	—	—	No flicker elsewhere
—	14·4	—	1°, 2°, 5°, and 10° flickering	—	—	15·7	1° only flickering
—	—	17·8	1° only flickering	18·8	—	—	No flicker elsewhere
—	15·3	—	All others flickering	—	13·3	—	All others flickering except 2°
17·6	—	—	? 10° still flickering; others not	—	—	15·6	1° only flickering
				—	12·8	—	
—	—	17·0	1° only flickering	—	—	15·6	1° only flickering. (More peripheral points also flicker)
—	14·5	—	All others flickering	17·0	—	—	No flicker elsewhere
					2° 12·0		All others flickering

avoid these factors. Thus, with the object at brightness 17·4 units, the speed of revolution of the 18-sector disc was increased until flicker was abolished everywhere except at 0° and 1°. After prolonged rest, no trace of flicker was detectable elsewhere even on momentary observation in the extreme periphery of the field of vision. In four other experiments the brightness of the object has been 25 units and we have confined our attention to three only of the fixation points. In the above tables, which give the results of these experiments, the temporal sequence of the observations is indicated. The notes refer to momentary transfer of gaze to other points while the experimenter was timing the revolutions of the disc. Central fixation, for which higher rates than any of those quoted would have been necessary, is disregarded in the notes.

The general features of the curves in Figs. 1 and 2 may now be regarded as established. The region of minimum sensitivity to flicker has nearly always lain between 2° and 3° from the fovea, but occasionally the lowest values have been obtained at 5°, especially with the more intense stimuli. Indeed, when the illumination has exceeded 130 units, the peripheral rise in the curve has sometimes been absent altogether. With weaker stimuli we have never failed to find it.

2. *Object surrounded by white.*

In this section will be reported a few observations made under somewhat different conditions from those which have hitherto been described. Immediately in front of the velvet curtain was placed an evenly diffusing white screen, 64 cm. × 51 cm., carrying black fixation marks at appropriate distances from the centre of a circular hole, 1·5 cm. in diameter, through which the test object could be viewed. The screen was illuminated by two powerful electric lamps placed behind, and to each side of, the observer's head. This illumination was kept constant throughout the experiments (including those in which the white screen was removed at intervals in order that the effect of the black velvet surround might be compared with that of the white screen). The illumination of the object was varied as before. When its intensity was about 16 of our arbitrary units and the disc was rotated rapidly enough to abolish flicker, screen and object appeared to the observer of approximately equal luminosity. The brightness of the screen was therefore about 8 units. (Unfortunately no exact determination of this figure was made.) The object thus appeared separated from the screen by a black ring about half the diameter of the object in width.

Determinations of fusion frequency were found more difficult under these conditions than when the surroundings were dark. The test patch was often difficult to see at all in indirect vision, and especially so at eccentricities exceeding 5° when the object was much less luminous than the screen. Even with central fixation it was sometimes difficult to state confidently the exact point at which flicker ceased. Nevertheless, certain quite definite conclusions can be drawn.

It was found by Schenck [1896] that a higher frequency of intermission is required to abolish flicker when the surroundings are white than when they are black. The matter has been very fully investigated by Lythgoe and Tansley [1929], who, for objects brighter than their background, obtained increasingly high frequencies with increasingly bright surroundings in all regions of the retina provided the illumination of the test patch (which subtended 1°) exceeded about 10 metre-candles. When the background was made brighter than the object, the frequency began to fall again. We shall consider their interpretation of the phenomenon later. Our findings with the very small object are in general agreement with these results.

In the experiments tabulated below, alternate observations were made with and without the white screen. Central fixation and a fixation point 5° to the right of the object were used. The luminosity of the white screen was about 8 units in all experiments, while that of the object when fused was half the value given for the physical intensity of its bright phase. The readings are given in flashes per second.

Bright phase intensity 2·8.

Observer *C*.

Fixation central		Fixation 5° peripheral	
White screen	Black velvet	White screen	Black velvet
25·5	—	13·8	—
—	20·6	—	11·8
21·8	—	—	—
—	19·3	—	—
22·9	—	—	—
—	18·9	—	—

Bright phase intensity 6·25.

Observer *C*.

Fixation central		Fixation 5° peripheral	
White screen	Black velvet	White screen	Black velvet
28·8	—	17·6	—
—	23·0	—	13·8
27·7	—	17·0	—
—	23·7	—	13·4
25·5	—	—	—
—	22·1	—	—

Bright phase intensity 35.
Observer *R*.

Fixation central		Fixation 5° peripheral	
White screen	Black velvet	White screen	Black velvet
31·8	—	25·0	—
—	28·5	—	19·4
28·9	—	26·2	—
—	26·9	—	19·4
28·9	—	27·7	—
—	27·1	—	21·2

Bright phase intensity 216.
Observer *C*.

Fixation central		Fixation 5° peripheral	
White screen	Black velvet	White screen	Black velvet
35·4	—	22·5	—
—	32·5	—	18·5
33·2	—	21·9	—
—	30·8	—	17·3
33·5	—	19·9	—
—	29·8	—	14·5

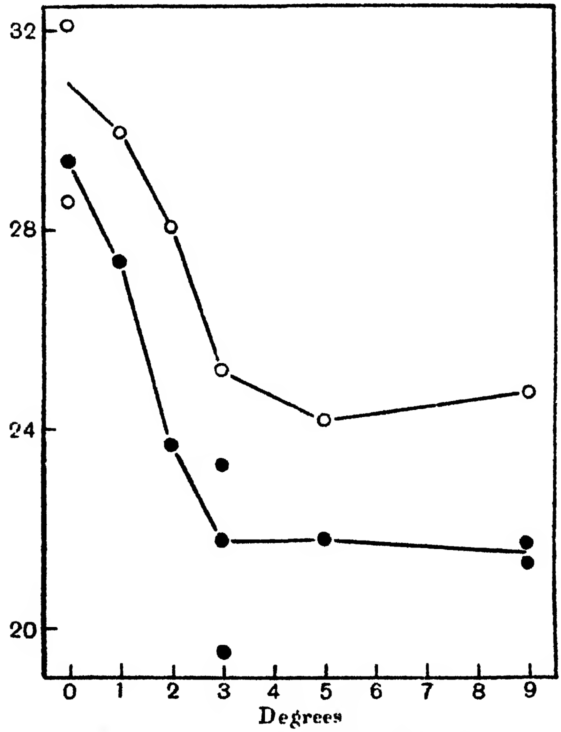

Fig. 4. Upper curve: observer *R*; brightness of object 6·25. Lower curve: observer *C*; brightness of object 22. Horizontal fixation points. As Fig. 2, except that object has white surroundings of luminosity 8.

The steady downward creep in each of these series is doubtless a phenomenon of adaptation—a factor difficult to control when the eyes are alternately exposed to light and to dark fields. In every case, when the disc was rotating just fast enough to abolish flicker with the black background, flicker at once appeared on replacing the white screen, and *vice versa.*

Curves relating fusion-frequency with region of retina stimulated (Fig. 4) resemble those obtained with black surroundings. Both show a rapid fall on passing outwards from the fovea, but, for a given luminosity of the test object, the former show higher readings at all points than the latter. The occurrence of a secondary rise beyond 3° is, however, very doubtful in these experiments. It has already been stated that determinations in the periphery are not easy. Our general impression is that the values for 3°, 5°, and 9° are identical.

Discussion.

As was explained in the introduction, the experiments which have been described were intended to minimize such areal effects as "spatial summation," which may be expected to vary in the extent of their occurrence in different regions of the retina. With this end in view the test object was made extremely small. We shall therefore ascribe the differences found between the foveal and peripheral parts of the retina to intrinsic properties of the receptors of those regions and their retino-cerebral pathways. For convenience of description we shall frequently neglect the afferent path and speak only in terms of rods and cones. But we do not thereby mean to imply that the receptors alone form the physical basis of flicker phenomena.

Both when the test object was isolated in a black void and when it was surrounded by a white screen, the critical frequency for extinction of flicker fell rapidly as the gaze was transferred from the object itself to points 2° or 3° away from it. Cones are believed to be the dominant receptor elements in this region (see Creed and Granit, 1928). We therefore conclude that the foveal cones require a higher frequency for fusion than do the peripheral, or alternatively that the increasing proportion of rods to cones accounts for the drop. Beyond 3° the fusion frequency changes little. In conformity with this, the structure of the retina becomes here more uniform, with rods preponderating. Chievitz [1889] found the ratio of cones to rods to be 1 to 15–18 all over the periphery of the retina, although their absolute numbers decreased considerably as one

approached the ora serrata. Other authorities are less definite and give the impression that the ratio becomes progressively smaller on passing outwards.

But if the peripheral parts of the retina are intrinsically less sensitive to flicker than the fovea, why is it that so many workers have found higher values for the fusion frequency of intermittent lights in indirect than in direct vision? The apparant discrepancy can hardly be because our tiny object only throws a sharp intense image on the fovea whereas optical imperfections make the image less sharp and dimmer in eccentric vision; for the fall is most marked quite close to the fovea. It is not a matter of the intensity of illumination, for in Sherrington's experiments [1904] the brightness of the object (subtending 2° 17′) must have been well within our range (probably 250–300 of our arbitrary units). Yet "an image would fail to flicker when received on the fovea that would distinctly flicker when its image fell just outside the fovea." Nor is it a matter of the method employed for producing the intermissions, for with a slightly modified technique we too have found flicker more marked in indirect vision with objects subtending 2°–8° visual angle at intensities between 0·01 and 17 units. In these experiments, when flicker has been abolished for fixation of the centre of the object, it has still been obvious when points from ½° to 10° from the centre have been tested. The object has sometimes appeared to flicker more at eccentricities between 4° and 7° than at points more central or more peripheral (cf. Lythgoe and Tansley, 1929, p. 61). We have not, however, investigated the last point carefully.

We know from personal observations as well as from the published work of others that increase in the size of a flickering object resembles increase in luminosity in that it causes marked increase in the fusion frequency [Bellarminow, 1889; Baader, 1891; Ives, 1912; Granit and Harper, 1930]. But Granit and Harper have also shown that this effect is much greater for images in the periphery than for images in the centre of the retina (as is to be expected from anatomical and other physiological considerations). The correct answer to the question propounded in the last paragraph appears then to be that with these larger objects interaction between neighbouring areas of retina becomes of importance. The excitation caused by one part of the image is added to that caused by other parts. This areal summation is so much more marked a property of the peripheral than of the central retina [Heinz and Lippay, 1928; Kleitman and Piéron, 1929; Granit and Harper, 1930] that its effects more than compensate for the

intrinsically lower fusion frequency of the former as compared with the latter[1].

The peripheral rise seen in many of our curves may well indicate that the same factor has been at work even with our minute object. At 20° the excitation caused by its image is probably converging on only about one-eighth as many ganglion cells as at 10° (perhaps on 6 instead of on 45). It may also be tentatively suggested that the absence of peripheral rise at high intensities and in the experiments with the white screen is due to summation having, under these conditions, already attained its limit.

Lythgoe and Tansley [1929] put forward a very different explanation of fusion frequencies higher in the periphery than in the centre. From their very thorough and careful experiments they were led to the conclusion that all "responses at high illuminations are due not to the rods but to the cones" and that "the critical frequency of the peripheral cones is higher than [that of] those of the fovea" (pp. 40–2). We agree with them in believing that the phenomenon cannot be ascribed to the peripheral rods (which may or may not be responding), but it seems clear from our experiments that over a wide range of "high" illuminations the foveal cones exhibit a higher fusion frequency than do any structures, whether rods or cones, in the periphery.

One other subject which merits brief discussion is the influence of bright surroundings on fusion frequency. Graham and Granit, in a recent paper [1931], have described the alterations which occur in the fusion frequency of a semicircular object, the radius of which subtends 1°, when an adjacent similar object is illuminated simultaneously. When the two were of equal brightness, the fusion frequency of the first was always raised by the presence of the second. With the brightnesses unequal, the fusion frequency of the darker was unaltered or lowered by the presence of the brighter. This they ascribe to retinal inhibition. It is interesting that no such effect was manifest in our experiments where, instead of a small adjacent object, a large area completely surrounding the test object was used. Indeed, the results recorded in the table above show, if anything, the reverse effect. With central fixation, the percentage increase in fusion frequency caused by the white screen was greater (viz. about 20 p.c.) with objects darker than with objects brighter than the screen (about

[1] The possibility of variation in the size of the pupil according to the region of retina stimulated, with consequent alteration of fusion frequency, should also be borne in mind. It seems highly improbable, however, that this factor should come into play with stimuli of large, but not with those of small, area.

9 p.c.). It is possible, however, that percentage increase is not a justifiable basis of calculation.

The simplest explanation of the effect of the white screen in raising fusion frequency would seem to be in terms of interaction between the structures excited by its image and those excited by the image of the object. Possible types of interaction are an increase in the effective intensity of the bright phases by spatial facilitation (summation) or an increased blackness of the dark phases by simultaneous contrast. The effect is more marked in the periphery (5°), where at three different intensities the fusion frequency was raised by 25–30 p.c., than at the fovea. The same is true of the striking examples of interaction studied by Granit and Harper [1930] and by Roaf [1931].

An alternative explanation is offered by Lythgoe and Tansley [1929], viz. that it is a phenomenon of adaptation and not one of simultaneous contrast or spatial induction (p. 37). They find the critical frequency for cones to fall during the course of dark adaptation, and suggest that the effect of luminosity of surroundings is to determine the general level of adaptation. This view seems hardly compatible with their finding that the critical frequency falls, not only on lowering the field brightness below, but also on raising the field brightness above, the illumination of the test patch (p. 34). Moreover, to account for the fusion frequencies tabulated in Part II of our results, changes in the level of adaptation would have to occur with surprising rapidity in both directions. It therefore seems preferable to postulate nervous interaction as part, at least, of the mechanism by which white surroundings raise sensitivity to flicker.

Summary.

1. The critical frequency for extinction of flicker in a white object subtending 12′ visual angle has been determined at various positions in the field of vision, viz. up to 20° from the fixation point in the horizontal meridian, and up to 10° in the vertical meridian.

2. Over a considerable range of brightness, so small an object flickers much more when its image falls on the fovea than when it is viewed eccentrically. The lowest fusion frequency occurs at about 2–3° from the fovea.

3. When the surroundings of the object are black, the fusion frequency rises somewhat on passing to regions more peripheral than this. This statement probably does not apply either at the more intense luminosities investigated or when the object is surrounded by a white screen.

4. It is argued that the foveal receptors and their central connections have a higher intrinsic fusion frequency than the peripheral, and that the apparently contrary results obtained with larger objects are due to spatial summation in the periphery.

5. The fusion frequency in all positions is raised by surrounding an object with a bright background. This is ascribed to interaction between the structures excited by the image of the test object and those excited by that of the background.

6. The generally accepted figure for the density of foveal cones, viz. 13,500 per sq. mm., is inaccurate. There are actually about 120,000 cones per sq. mm. in the human fovea.

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LAPICQUE'S CANONICAL STRENGTH DURATION CURVE.

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INTRODUCTION.

EVER since the study of the strength duration curve was initiated by the researches of Hoorweg and of G. Weiss, numerous investigations have been undertaken to obtain an exact experimental relation between the liminal intensity of a stimulating current, and the duration through which this current flows. The object of most of this work has been to test experimentally some physical theory of excitation, and the excitable tissue usually chosen has been the frog's sciatic nerve. Now with the theoretical aspect we are not here concerned, but the consideration of the tissue employed has given rise to an important generalization due to Lapicque. This investigator has worked upon excitable tissues of very diverse kinds, each of which has its own peculiar strength duration curve, but he claims that if the curve for each tissue is suitably scaled it will coincide with a fixed curve identical for all tissues and called by him "canonical."

The canonical curve relating i and t may be represented within experimental limits by the equation

$$= a\sqrt{\frac{t+\theta+\sqrt{(t-\theta)^2+0\cdot16\theta^2}}{2t}} \qquad .(1),$$

where i = current, t = time, a = rheobase, and $\theta = 3\cdot8$ times the chronaxie.

This equation is not based upon any physical considerations nor is it claimed to have any physical significance, it is purely given as a compact expression from which one may compute the relation which Lapicque has found experimentally. If this relation is true for all excitable tissues then any satisfactory theory of excitation must allow it to be deduced

(approximately at least) from the premises, and this was the chief value to the exact form of the canonical curve, until the last year or so.

Quite recently the investigations of Lapicque and of myself have emphasized a phenomenon demonstrated earlier by Lucas and others [Lucas, 1907–8]. As a result it is quite clear that a muscle may exhibit two strength duration curves of very different time constants (? chronaxies), and the question arises as to their significance. Now according to Lapicque's theory of isochronism, the chronaxie of a muscle fibre is the same as that of its motor nerve provided that the two tissues are in physiological connection, not otherwise. In degeneration, curarization (by curare, not by strychnine), and fatigue, the muscle chronaxie is much prolonged. But of the two excitabilities which may be found in muscle by excitation through fluid electrodes of the "block type" [Rushton, 1930] one has a chronaxie the same as the motor nerve, and the other is very much longer. (The former is called the γ excitability, the latter the α, after Lucas.) In view of the isochronism theory therefore, it might be supposed that the γ fibres are normal fibres in connection with their nerves while the α fibres are physiologically severed. The second part of this suggestion however is not correct. I have made a large number of experiments of different kinds to test whether the α fibres are physiologically normal, and I can find no evidence to the contrary. In brief, the α effect may be obtained from spinal preparations, from those freshly excized, and those after 24 hours' equilibrium with Ringer's fluid [1930]; it was found in all of a dozen different muscles investigated from various parts of the frog, and the isometrical twitch was barely distinguishable from that due to indirect excitation when the two curves were superimposed [1931 *a*]; finally the α fibres were shown not only to be supplied by nerves, but to be practically the only fibres that are supplied, for their contraction accounts for nearly all the tension time developed in a maximal twitch excited through the nerve [1932].

It therefore seems to be legitimate to conclude that the α fibres are not abnormal, but this forces us to an important conclusion with regard to the theory of isochronism. For either the α curve is not a "true" strength duration curve from which a "true" chronaxie can be found, or else the great difference between the α chronaxie and that of the nerve which supplies these fibres completely falsifies that theory.

Lapicque accepts the first alternative [1931] and rejects the validity of the α curve on account of the fact that in general it does not fit the canonical curve within the limits of experimental error (which in my experiments are 5 to 7 p.c.). Here now is a new and very important use

for the canonical curve, namely to distinguish true chronaxies from false. For instance, if the chronaxie of a patient be sought clinically (by excitation through the skin after the manner of Bourguignon [1923]), the way to learn whether the value obtained is the "true" one, or whether it is in error by about a hundredfold (as in the case of the α curves) is to map out the whole curve and see whether this is canonical or not. Thus not only in theory but in urgent practice is the canonical curve of the first importance, if we follow Lapicque.

Now neither Lapicque nor anyone else to my knowledge has obtained a strength duration curve from vertebrate skeletal muscle or nerve that follows within even moderate limits the canon at short

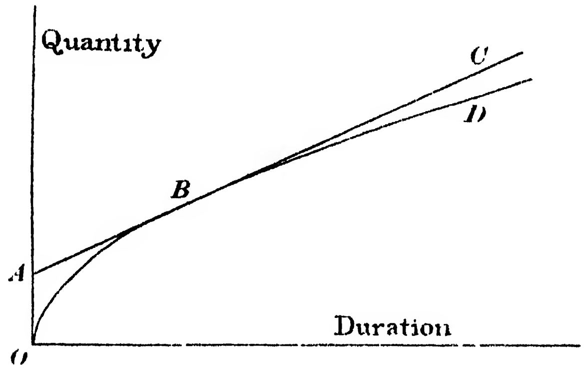

Fig. 1. Quantity-duration curves. *ABC* Weiss straight line. *OBD* Lapicque parabola at short durations.

durations. It is well known that the experimental results of G. Weiss plotted as quantity of electricity against duration, fell approximately upon the straight line *ABC*, Fig. 1. But Lapicque's canonical curve at short durations coincides with the parabola *OBD*[1], hence the results of Weiss were clearly not canonical. As Lapicque points out, the best experimental determinations do not follow the Weiss line exactly but at short durations they drop below it, but they do not drop nearly sufficiently to become canonical. This discrepancy is well appreciated by Lapicque who attributes it to physical imperfections of the stimulating circuit [1926, pp. 89, 116], and who considers that the only reliable curves are those obtained from slowly reacting muscles [p. 97] (*e.g.* snail's foot, frog's stomach, etc.), and in spite of the great physiological

[1] For when t is small compared with θ equation (1) reduces to $i\sqrt{t}=\text{constant}$.

difficulty attendant upon obtaining repeatable results from such tissues [p. 77] it is from these that the canon has been formulated.

The chief physical error which is supposed to account for the deviation of the experimental results from the canon in the case of frogs' nerves is inductance. It is certainly a fact that an inductance placed in series with the tissue will cause the current instead of rising abruptly (Fig. 2), to its steady value, to rise gradually along an exponential (broken line), and hence if the current ceases at AB after a very short duration, the actual current which has passed OBA will only be a fraction of the calculated rectangle $ODCA$. It is clear, then, that inductance, if present to an appreciable extent in this way will account qualitatively at least for the divergence of the experimental results from the canonical curve.

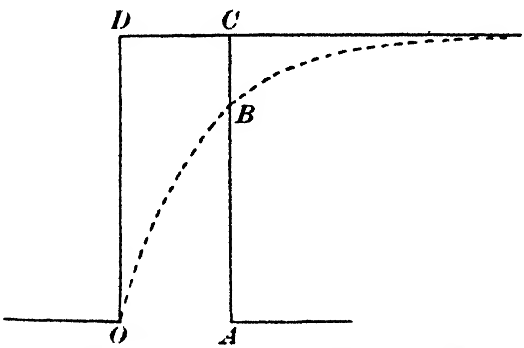

Fig. 2. The effect of inductance upon a "rectangular" stimulus. Ordinates: current, abscissæ: time. $ODCA$ non-inductive stimulus, OBA, inductive stimulus.

Lapicque, however, describes [p. 296] the care he has taken to avoid inductance by employing resistances of the type "crayons Conté," and with this arrangement it is certainly surprising to learn that inductance produces deviations appreciable still at $0{\cdot}3\sigma$ [p. 215].

But as a matter of fact his method of proving that his circuit is highly inductive is erroneous. In order to test the matter he arranged a double switch so that either the tissue or a ballistic galvanometer could be substituted in the stimulating circuit [p. 111]. In the first case he measured the threshold, in the second (by the fling of the galvanometer) the quantity of current passing, and found that, for short durations, the quantity was much less than that calculated from the product it [p. 116]. This shows that when the galvanometer was in the circuit, the circuit was inductive, which was to be expected in view of the inductive nature of the galvanometer. We have no information concerning the inductance

of the circuit when the tissue is substituted, which is in fact the circuit which concerns us. It seems very likely, from its construction, that that circuit was quite non-inductive within experimental limits, and that Lapicque's results, like those of other workers, were essentially accurate —but uncanonical.

In order to test this matter definitely I have attempted to obtain the strength duration curve from the frog's sciatic nerve with a stimulating circuit relatively free from those physical imperfections which might account for the uncanonical nature of the results.

We proceed to a detailed consideration of the apparatus.

Description of apparatus.

Pendulum.

This was the original short range foot-pendulum constructed by Keith Lucas and described by him [Lucas, 1907]. The adjustment of

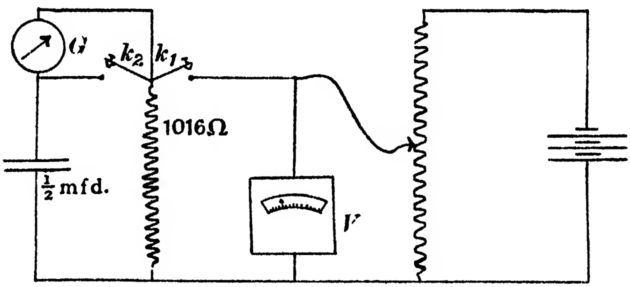

Fig. 3. Circuit for pendulum calibration. k_1, k_2, keys of pendulum; G, ballistic galvanometer; V, voltmeter.

the contacts was controlled by a fine screw and viewed through a micrometer eyepiece, settings could be made to 0·01 mm. Since the speed of the pendulum was about 6 mm. per σ, the error in the setting of the scale could not amount to more than $\pm$ 0·001σ.

With regard to other errors, such as variations of settings of contacts for a fixed position of scale, vibrations of the apparatus and other fortuitous fluctuations in the motion of the arm, we proceed to an experimental investigation from which emerges the remarkable fact that these errors lie within $\pm$ 0·003σ, which may be taken as the limits of accuracy of the instrument.

The method of calibration was similar to that described by Lapicque [1926, p. 335] and Hozawa [1930]. The method differs from Lapicque's

in that his nerve muscle preparation is replaced by a ballistic galvanometer, which could be read to an accuracy 20 times as great as the limits of threshold measurement of a nerve. The method differed from that of Hozawa in using k_2 not as a series key to stop the condenser discharge, but as a short circuit across the galvanometer (Fig. 3) which avoids errors due to leaks in the condenser and adjacent circuit. The condenser was a standard half microfarad with resistance between the plates greater than 10^{10} ohms. The resistance in the discharge circuit was a Ferranti wire-wound non-inductive resistance of 1016 ohms.

Thus if D_o is the galvanometer deflection when k_2 is opened before k_1, D_t is the galvanometer deflection when k_2 is opened at time t after k_1,

$$\begin{aligned} t &= CR \log \frac{D_o}{D_t} \\ &= 0{\cdot}508 \log \frac{D_o}{D_t} \quad \text{in } \sigma \qquad \ldots\ldots(2). \end{aligned}$$

When k_2 was left closed and k_1 opened there was no deflection showing that k_2 was an adequate short circuit. The galvanometer deflections could be read correct to 1 mm. of scale, and successive repetitions seldom diverged by more than 2 mm., D_o being always made about 440 mm.

To estimate the error which this divergence produces in the calculated time interval, we obtain from (2) by differentiation

$$\Delta t = -\,0{\cdot}508 \frac{\Delta D_t}{D_t} \quad \text{in } \sigma,$$

where

$$\Delta t = \text{error in interval},$$

$$\Delta D = \text{error in deflection } D_t.$$

But if $$D_t > 200, \quad \frac{\Delta D_t}{D_t} < \pm\, 0{\cdot}5 \text{ p.c.}$$

Therefore t alters by less than $\pm\, 0{\cdot}0025\sigma$.

D_t here corresponds to intervals less than $0{\cdot}4\sigma$. It may similarly be shown that the error is less than $\pm\, 0{\cdot}005\sigma$ for intervals less than $0{\cdot}8\sigma$.

It was possible that the calibration curve might alter from day to day to an extent outside the above limits, and to examine this point I made a calibration almost every day for a month. It was found that there was no appreciable change in the curve except for the zero setting. The motion of the lever was uniform, but the exact setting of the steel rods which served as contacts altered from time to time, but never more than $0{\cdot}01\,\sigma$, and when once the alteration occurred, values remained fixed with the new zero. To take this into account however I made a few observations both before and after each strength duration curve, and thus found the

zero for that case and verified that it had not changed during the experiment.

In view of these observations, we have the rather surprising result that it is possible to regulate the passage of a current within limits of $\pm 0{\cdot}003\sigma$, and even less for very short intervals. Lapicque is very sceptical about the accuracy of pendulums, and I shared his views until the present investigation convinced me of the excellence of this instrument of Lucas's.

The foregoing considerations justify us in regarding the pendulum as accurately repeatable within the specified limits, but they do not justify

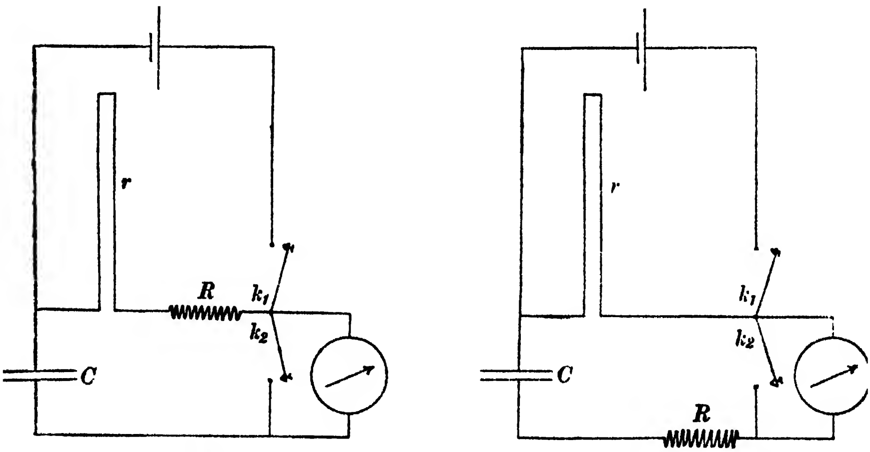

Fig. 4 (*a*) and (*b*). Circuits for estimating effect of inductance on pendulum calibration (see text).

us in accepting the calculated value of t in σ without further discussion. If the standard capacity was not exactly half a microfarad all the results would be altered in the same ratio. Since however (from the canonical equation) this would only be equivalent to altering the temperature of the tissue by a very small amount it does not affect the comparison which is to be made between experiment and canon, and in any case the error in the unit of time will be small. If, however, the resistance is inductive the calculated value t will not be proportional to the interval, but it will be some complicated function of it. It is necessary therefore to estimate the error due to the inductance and if necessary to correct for it. This has been done as follows.

A piece of wire 2 metres long with resistance 20 ohms ran straight

for a metre then doubled on itself and ran back for a metre. The inductance of this is negligible. This resistance is placed as shown at r, Fig. 4 (a) and (b), the 1016 ohms being changed in the two cases as shown at R.

In (a) the current initially flows through R and hence tends to persist owing to inductance when k_1 is opened, and this increases the rate of discharge of the condenser at first. In (b) there is no current initially in R and hence the inductance delays the discharge of the condenser at

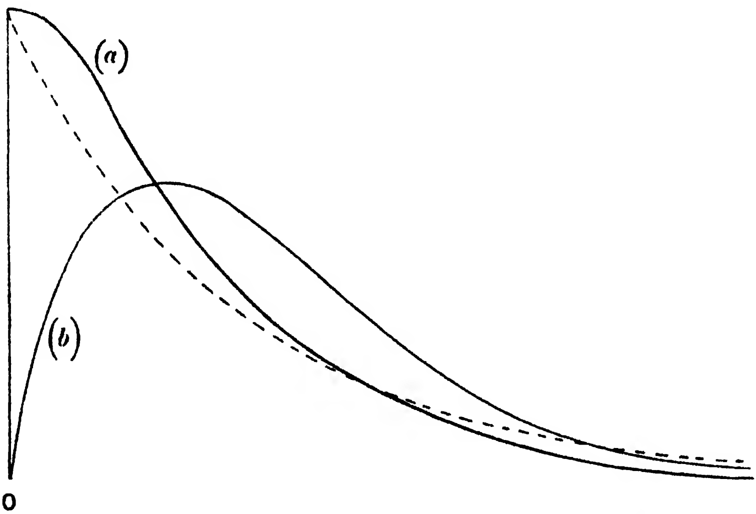

Fig. 5. Current flowing from condenser (ordinates) at various times (abscissæ) after O, the instant when k_1 (Fig. 4) is opened. Curves (a), (b) correspond to R being highly inductive, Fig. 4 a, 4 b respectively; broken curve (exponential) corresponds to R, being non-inductive in either case.

first. The non-inductive case therefore lies initially between these two. Fig. 5 shows current through the condenser plotted against time in the case of (a) and (b) when R is highly inductive. As the inductance diminishes so both these curves approach the broken line, finally to coincide with it in the ideal inductionless case.

Now the fling of the galvanometer which we measure is proportional to the charge remaining on the condenser at the moment when k_2 is opened, which is proportional to the area of the curve in Fig. 5 to the right of the ordinate corresponding to this moment, or to Q_o minus the area to the left of this ordinate since the total area of each curve is Q_o, the initial charge on the condenser.

Thus if the inductance were zero

$$t_a = CR \log \frac{D_o}{D_t} \quad \text{for circuit } (a)$$
$$= t_b = CR \log \frac{D_o}{D_t} \quad \text{for circuit } (b)$$
$$= t = \text{true interval in } \sigma.$$

But since the curves (*a*) and (*b*), Fig. 5, deviate from the exponential (broken line) one on either side, initially, we have the relation

$$t_a > t > t_b \qquad \ldots\ldots(3),$$

or the error in using t_a as the true value of the interval $< (t_a - t_b)$ for small values of t.

The experiment was easily carried out by arranging two switches to transform circuit (*a*) into (*b*) and back, so that t_a and t_b could be compared without delay for any setting of the pendulum. It was found that $(t_a - t_b)$ was never more than $0{\cdot}005\sigma$ and hence lay within the limits of error of the apparatus. However, a more exact analysis of the equations for current flow in these two cases shows:

(i) That t_a is a much closer approximation than t_b to the true interval t (as is easily appreciated from Fig. 5).

(ii) That the error in assuming that t_a is the true interval for any setting of the pendulum whatever, is less than $2\,(t_a - t_b)^2$ measured for any one setting of the pendulum (greater than $0{\cdot}1\sigma$), all times being expressed in σ. Thus in the present case, the calibration error due to inductance

$$< 2\,(0{\cdot}005)^2 = 0{\cdot}00005\sigma.$$

As a check upon the method I placed in series with R a very large and heavy self-inductance of about 0·06 henry (calculated from D.C. and A.C. resistance measurements), and measured $(t_a - t_b)$ for values of t_a of about 0·1, 0·4, and $0{\cdot}7\sigma$. The results all agreed in giving

$$(t_a - t_b) = 0{\cdot}067 \pm 0{\cdot}003\sigma.$$

The inductance required by calculation to produce this value is 0·056 ± 0·003 henry, which is in good enough agreement with 0·06 from direct measurement, since the calculation employed is only strictly true for small inductances.

We may therefore conclude that the expectations from the electrical theory are realized experimentally, and that the calibration of the pendulum by the circuit (*a*) (which is that originally described, Fig. 3) suffers from no appreciable error due to inductance, and that this instru-

ment, freshly checked with regard to zero error, is accurate to $\pm$ 0·003σ for intervals less than 0·5σ and to $\pm$ 0·006σ for intervals up to 1σ.

Tissue and electrodes.

The tissue investigated was the sciatic nerve of the frog with gastrocnemius attached to serve as index of the efficacy of excitation, the muscle being observed directly. The preparation was sometimes at room temperature and sometimes cooled with ice in order to allow of a more exact examination of the durations short compared with the chronaxie. The electrodes were silver wires freshly coated with chloride electrolytically. The nerve was placed on these in air in a moist chamber, and excited by a descending current, through 15-20 mm. of its length. The question of the canonical curve is closely related to that of the nature of the electrodes; the form here employed is that which Lapicque recommends.

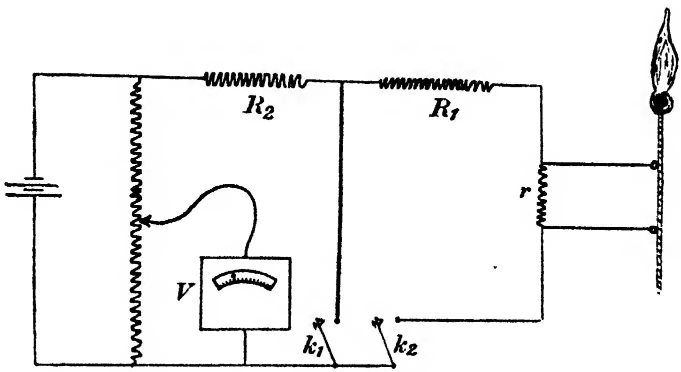

Fig. 6. Stimulating circuit for strength-duration measurements. V, voltmeter; k_1, k_2, keys of pendulum.

Stimulating circuit.

Lapicque attributes serious errors to the inductance present in stimulating circuits, and care must be taken to overcome these objections. Consider the circuit Fig. 6 and suppose at first that R_1 alone is inductive, then (as we saw earlier, Fig. 2) when k_1 is opened the current in R_1 (and hence in the nerve) will rise gradually to its final value (curve 1, Fig. 7). If on the other hand R_2 alone is inductive, on opening k_1 the large current which initially flowed through it will tend to persist and only gradually decline, hence the current through the tissue will fall to its final value

(curve 2, Fig. 7). If both R_1 and R_2 are inductive these two tendencies oppose each other and the current in the tissue rises if the ratio

$$\frac{\text{inductance}}{\text{resistance}}, \left(=\frac{L}{R}\right)$$

is greater in the circuit to the right of k_1 than to the left. If the two ratios $\frac{L}{R}$ are equal, a perfect rectangular wave results, behaving as though no inductance at all were present (curve 3, Fig. 7). These statements may easily be verified from the inspection of the well-known equations for current flow in an inductive circuit.

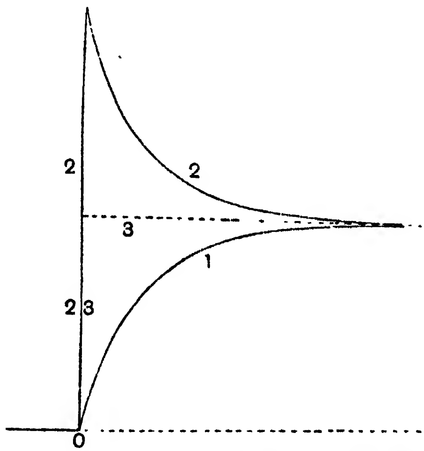

Fig. 7. Current flowing through tissue (ordinates) at various times (abscissæ) after O, the instant when k_1 (Fig. 6) is opened. Curve 1 is when R_1 alone is highly inductive, curve 2 when R_2 alone is. The perfect rectangular curve 3 is when R_1 and R_2 are both inductive to the right extent (see text).

As a result of these considerations it is clearly possible in theory to balance out the effect of inductance entirely by means of a fixed inductance in one half of the circuit and a variable one in the other, but in practice the difficulty is to recognize the balance point. On this account I have not attempted an exact balance, but have employed R_1 and R_2 as non-inductive as possible to reduce the effect of inductance to small dimensions in any case, and have then arranged the rest of the circuit so that the residual inductive effect will act in the direction opposite to that which might explain the uncanonical nature of the observed results. The detailed description will be postponed until these results have been presented.

In the actual circuit used for the experiment, the tissue was shunted

by 200 ohms and R_1, R_2 were each wire-wound non-inductive resistances of 1000 ohms, being the ratio arms of a Wheatstone bridge. Current strengths were varied by a potentiometer and the E.M.F. led off was read directly by an accurate voltmeter. By means of a switch (not shown in the diagram) the pendulum could at any moment be connected to the calibrating circuit (circuit a) so that the interval for any setting in case of doubt could be redetermined without delay or shift of setting. The reliability of the pendulum however rendered this hardly necessary.

Experiment and results.

Experiment.

The preparation was set up on the electrodes and allowed to rest (and cool when necessary) for about an hour. During this time the pendulum zero was tested. The rheobase and chronaxie were then taken and thresholds for a number of durations less than the chronaxie obtained. The measurements were then repeated in the reverse order, and when the divergence was small the experiment was considered satisfactory. At the end of the determinations the pendulum zero was again tested.

Results.

All the results that I obtained without exception gave a large divergence from Lapicque's canonical curve at short durations, just as do the results of former workers.

The canonical relation is given by Lapicque in the form quoted on the first page of this paper (1). This may conveniently be developed by Taylor's theorem into the following power series:

$$\frac{i\sqrt{t}}{a} = 1 + 0{\cdot}0046\left(\frac{t}{\tau}\right) + 0{\cdot}0021\left(\frac{t}{\tau}\right)^2 + \dots \qquad \dots\dots(4),$$

where $t =$ duration, $\tau =$ chronaxie,

whence it immediately appears that for durations less than τ,

$$i\sqrt{t} = \text{constant (correct to 0·5 p.c.).}$$

In the following tables the first column gives the duration of the current, the second gives the limits between which the threshold lay, while the third gives the repetition of the measurements in the reverse order. The fourth column gives the threshold calculated from the formula

$$i\sqrt{t} = \text{constant},$$

where the constant is adjusted to fit the results in the neighbourhood of the chronaxie.

At room temperature.

Duration in σ	Threshold: Observed		Calculated
Frog I			
∞	14 - 13·4	13·4- 13	—
0·175	31 - 30	30 - 29	30·0
0·11	47 - 45	45 - 43	37·6
0·07	65 - 62	63 - 60	47·3
0·032	115 -110	115 -110	70
0·012	>140	>140	116
Frog II			
∞	5·0- 4·8	5·2- 4·8	—
0·295	11·2- 10·6	11·7- 11·2	11·2
0·175	14·5- 14·0	15·0- 14·5	14·5
0·106	23 - 22	22 - 21	18·7
0·07	32 - 30	31 - 29	23·0
0·035	55 - 50	55 - 50	32·5

Cooled in ice to near zero.

Duration in σ	Threshold: Observed		Calculated
Frog III			
∞	5·4- 5·0	6·0- 5·6	—
0·98	13·5- 13·0	14·0- 13·5	13·5
0·50	23 - 22	23·5- 22·5	19·3
0·31	36 - 34	36 - 34	24·3
0·195	53 - 50	55 - 52	31
0·12	83 - 78	83 - 79	40
0·08	107 -102	115 -110	47
0·065	>132	>132	53
Frog IV			
∞	10·5- 10·0	10·5- 10·0	—
0·82	19 - 18	19 - 18	18·5
0·50	27 - 26	28 - 27	24
0·31	40 - 38	40 - 38	30
0·195	62 - 59	60 - 57	38
0·12	92 - 88	94 - 90	49
0·08	>130	>130	59

The threshold measurements are seen to be repeatable usually within the limits of 5 p.c., but the calculated values diverge from these by even a hundred p.c. at the shortest durations with cooled preparations. It is not necessary to add to these examples which are typical of all my observations and which only confirm those of former investigators. The deviation from the canonical formula is progressive and extensive and it merely remains to review the apparatus in order to see whether this can in any way account for the divergence.

Possible errors.

If the inductance of the circuit is to explain the results, we have seen that the ratio $\frac{L}{R}$ must be greater in the circuit to the right of k_1 than to the left, Fig. 6; but actually the reverse is the case. For if the two resistances R_1, R_2 are identical in resistance and inductance, then $\frac{L}{R}$ for the circuit on the right is lowered by the electrode system which is non-inductive, and even capacitative (assuming the tissue shunt non-inductive), whereas in the other circuit the potentiometer is inductively wound on an iron frame. Thus $\frac{L}{R}$ is greatest in the circuit on the left unless this is compensated for by $\frac{L}{R}$ being greater for R_1 than for R_2. This however is not the case for otherwise interchanging R_1 and R_2 would certainly make $\frac{L}{R}$ greatest in the circuit on the left. But in experiments to test this point, R_1 and R_2 could be interchanged by a switch and no alteration of threshold was ever observed even at the shortest durations. Thus the uncanonical results obtain for either position of R_1 and R_2 and hence cannot be explained by inductance, unless it be due to the tissue shunt. But inductance in this shunt will cause it to have a higher equivalent resistance for the shortest durations, and since increasing the shunt resistance was found to lower the threshold, the effect of inductance here also would be to increase the observed discrepancy, not to explain it. Exactly the same result follows from the supposition that the electrodes are polarizable, for that also favours the shortest durations and hence this defect would produce a threshold relatively too low at short durations, whereas what we find is precisely the contrary.

Conclusions.

The pendulum we have already considered at length, the circuit we have just reviewed, the experimental figures speak for themselves. Whatever may be the case with slow tissues, the frog's sciatic nerve does not fit Lapicque's canonical curve, and hence the canon loses its significance.

We are thus forced back upon the dilemma with which this paper opened. The α fibres are supplied by nerves whose chronaxie is about a hundred times as short as that of the muscle. Either Lapicque's theory of isochronism cannot be accepted or else the α curve is not a "true" strength duration curve from which a "true" chronaxie can be

found. Lapicque accepts the second alternative, and considers that the α curve is false because it does not fit the canonical curve. But in the present paper we have seen that this same objection applies to the strength duration curve of the sciatic nerve, obtained with Lapicque's type of electrodes, and hence all the chronaxies which have hitherto been determined by this method upon this classical tissue are also false. In particular, the experiments from which Lapicque derived his theory of isochronism are to be rejected for there also the sciatic nerve was used, and only a false chronaxie obtained.

It is very far from my wish to minimize the great service that Lapicque has done physiology by pointing out so forcefully that the manifest differences between fast and slowly reacting tissues are essentially mere changes in the unit of time appropriate for each tissue; but when he claims that this phenomenon is mathematically exact [1926, p. 76, footnote], and that the time scale is the only relevant difference between tissues of very varied structure and function, then his theory becomes improbable *a priori*, and untenable experimentally. Lapicque's results with slow tissues accord well with his canonical formula, his results at short durations with skeletal frog's nerves do not. This discrepancy he attempts to explain by inductance; but the present paper shows that inductance is not responsible and that frogs' nerves appear to follow at short durations a curve different from that of slow tissues.

Lapicque's canon is therefore an idea without adequate experimental support. It does not apply to the tissue which allows of the most accurate investigation, it has no theoretical backing or significance, it is not even very easily computed or appreciated mathematically (as compared for instance with the formula of H. and E. Lassalle [1928][1], which is numerically identical with Lapicque's within experimental limits). As a means of extricating the theory of isochronism from the dilemma with which it was faced, the canon is hardly more successful, for, as we have seen, it ends by destroying the validity of the very experiments upon which that theory was based. Thus though Lapicque's qualitative generalization is of great importance, his canon appears to have little utility.

But if Lapicque's canon has lost its significance how shall we tell "true" chronaxies from "false," seeing that such different values may

[1] $i=\frac{a}{\sqrt{2}}\sqrt{\frac{T}{t}+\frac{t}{T}}$, where $T=7\cdot78$ times the chronaxie and the other symbols are as in Lapicque's formula.

be obtained depending upon the electrodes employed? The remedy lies with anyone who cares to suggest a new criterion, but it is difficult to see why one value should be taken in preference to another. It seems to the writer much more satisfactory at this stage to avoid the complications which arise when we imagine that certain values of the chronaxie are "truer" than others. One thing is certain; until we understand more exactly the relation between the nature of the electrodes and the chronaxie obtained by them, it is essential to describe the first when giving an intelligible value for the second, and in this way the chronaxie becomes, not characteristic of the tissue alone, but of the tissue and the electrodes, as Davis insisted in 1922. Lapicque, however, wishes to restrict the term chronaxie to "true" chronaxies, and other values he calls "pseudo-chronaxies" [1931]. This nomenclature seems unfortunate especially since at present there is no method of distinguishing chronaxies from pseudo-chronaxies, but since Lapicque was responsible for the name in the first place it clearly should be used in the sense which he wishes.

It is therefore of practical importance to have a new name to distinguish the time constant of a strength duration curve, quite apart from any theoretical considerations as to the "truth" of this curve, and while realizing that this constant may not suffice to determine the whole curve. Since Keith Lucas used the term Excitation Time in just this sense, it may conveniently be adopted as this characteristic of any strength duration curve whether "true" or not. According to this proposal, therefore, the chronaxie is the particular Excitation Time in the case where the strength duration curve is canonical, or where it satisfies any new criteria which Lapicque may in future suggest.

SUMMARY.

Lapicque has recently insisted that unless the strength duration curve of a tissue coincides with an empirical curve which he calls "canonical," the chronaxie obtained from it will be "false," and may not be used in relation to his theory of isochronism which only applies to "true" chronaxies.

In the present paper it is shown that the chronaxie of the frog's sciatic nerve obtained by Lapicque's method of excitation is "false," for the deviation from the canonical curve is very great at short durations, and this is not due to inductance (as Lapicque supposed when he obtained the same results) for in the present case this and certain other

possible errors have been controlled. Thus Lapicque's definition of a "true" chronaxie apparently destroys the significance of all the work he has done upon the sciatic nerve since there he was only dealing with "false" chronaxies, and in particular this applies to the experiments underlying his theory of isochronism.

Since Lapicque wishes to restrict the name "chronaxie" to "true" chronaxies, it is important to have a new term which can be applied to any strength duration curve "true" or "false." Lucas's "Excitation Time" was used in just this sense and it is proposed that it be adopted.

I am indebted to the Government Grants Committee of the Royal Society for enabling me to obtain some of the apparatus used in this research.

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THE EFFECT OF LENGTH ON THE RESTING METABOLISM OF MUSCLE.

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During a study of the thermo-elastic properties of muscle (see the following paper), using the sartorius of *R. temporaria*, I noticed that a permanent deflection of the galvanometer occurred in the "heating" direction when the muscle was kept stretched. On release the "heating" disappeared. This observation was regarded at first with suspicion as possibly originating in some error, and various controls were made to prevent if possible its occurrence. These, however, all failed to do so. Further experiments soon demonstrated beyond doubt that in the case at least of English *temporaria* there is a genuine relation between the resting metabolism of a muscle and its length. This paper reports these experiments and similar ones on Dutch and Hungarian *R. esculenta*.

Fig. 1 shows the original observation. A pair of sartorii of *R. temporaria* were mounted on a thermopile and loaded with 100 g. A progressive rise of heat rate occurred, reaching a maximum, then settling down to a lower level which, however, was considerably higher than the original resting one. On release, after a sharp kick in the heating direction —probably a thermo-elastic effect—the galvanometer returned to—or as was more often the case, nearly to—where it was before the stretch. When the return of the galvanometer was incomplete after release, there was also incomplete restoration of the length of the muscle. Fig. 2 shows a more conspicuous case obtained in later experiments and illustrates the effect of increasing and decreasing the load by successive steps of 10 g. All observations were made at 20° C., unless otherwise stated.

The following sources of error may be considered:

(1) *Creep of galvanometer zero.* This may be dismissed immediately, for the Downing moving-magnet galvanometer employed usually kept its zero within 2 mm., at the sensitivity employed, throughout the whole day. Moreover, one cannot conceive that the creep should always coincide with the stretch and the release of the muscle.

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(2) *Stretching a muscle may change its contact with the thermopile surface.* The muscle was stretched by weighting one end of an isotonic lever, pivoted in its middle, whose other end was connected to the muscle sometimes by a thread, sometimes by a wire, over 2 ft. long. The amount of stretch was seldom more than 1 cm., usually about 5 mm. The lateral displacement of the muscle, therefore, was negligible. An important result of stretch is that the muscle becomes thinner and narrower and consequently less of it lies on the thermopile. This, however, other things

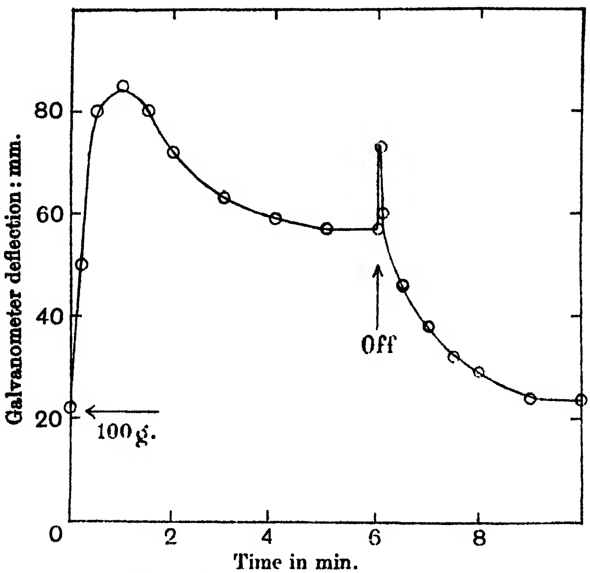

Fig. 1. Sartorius of *R. temporaria* in O_2 at 15° C., showing prolonged heat production while the muscle is kept loaded with 100 g. On unloading, the heat rate falls to the original resting level.

being equal, and, for a given rate of heat production, would cause a decrease and not an increase of the galvanometer deflection. If the face of the thermopile were slightly irregular, the muscle would lie more intimately in contact under capillarity with a low than with a high tension: with a high tension it would tend to bridge over a gap. The worse contact with the higher tension would diminish the deflection for a given heat rate, not increase it.

(3) *There might be insufficient equalization of temperature, and a hotter portion of the muscle might be brought into contact with the thermopile when it was stretched.* This is most unlikely, since the constant temperature

bath employed regulated within 1/500th of a degree. Furthermore, observations made in Ringer's solution, unstirred or vigorously stirred, gave results entirely similar to those in a gas.

(4) *Stretching the muscle might cause twitching,* either directly (by acting as a stimulus) or by some attendant irritation, such as rubbing of the muscle against thermopile or electrodes. This is made very improbable by the permanency of the increased rate of heat production

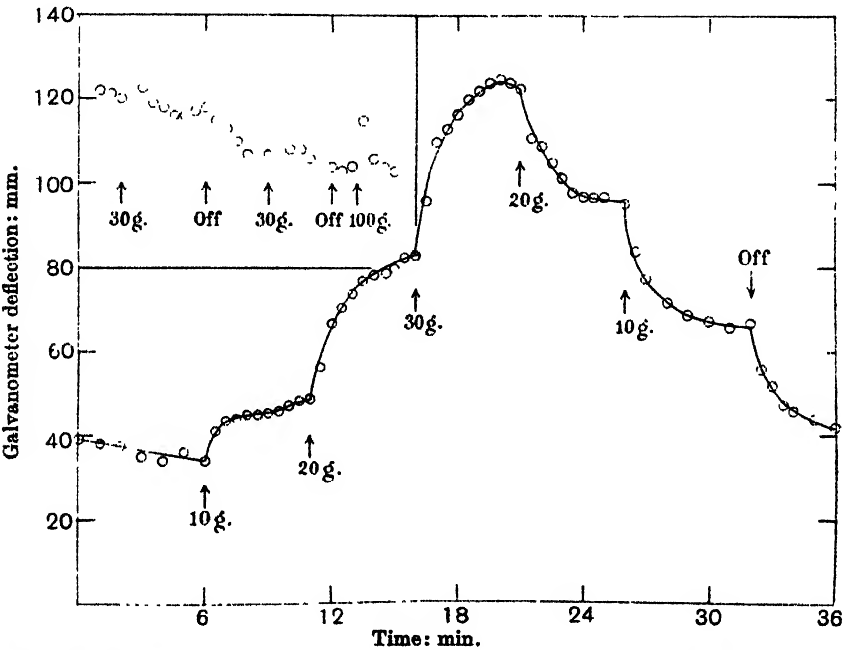

Fig. 2. Sartorius of *R. temporaria* in O_2 at 20° C., showing the effect on resting heat rate of loading and unloading the muscle by 10 g. steps. Inset showing the disappearance of the effect after the muscle was electrocuted.

following a stretch. Curarization, moreover, or rendering the muscle inexcitable by increasing the KCl content of the Ringer's fluid to about five times its normal amount or by soaking in isotonic sugar solution, all failed to abolish the phenomenon.

(5) Finally, in an *electrocuted or chloroformed muscle* stretching no longer causes any rise of its resting metabolism. This will be discussed more fully later: it is quoted here merely to show that the phenomenon is not due to extraneous error.

Its existence being certain, attempts were made to gain some insight

into the nature of the phenomenon which, for want of a better name, will be referred to as the "stretch response." If the increment of resting heat rate due to stretch be of the same nature as the original resting heat itself, it is to be expected that, for a given stretch, (*a*) it should be greater in oxygen than in nitrogen and (*b*) that if the stretch were done in nitrogen

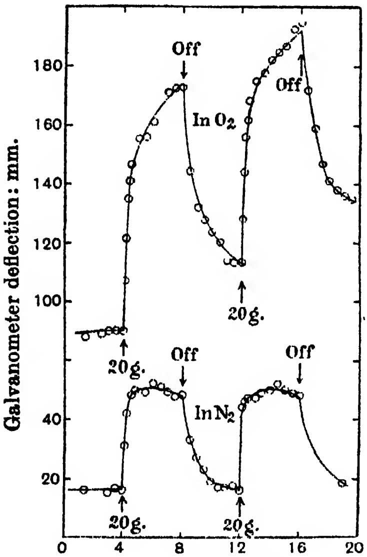

Fig. 3.

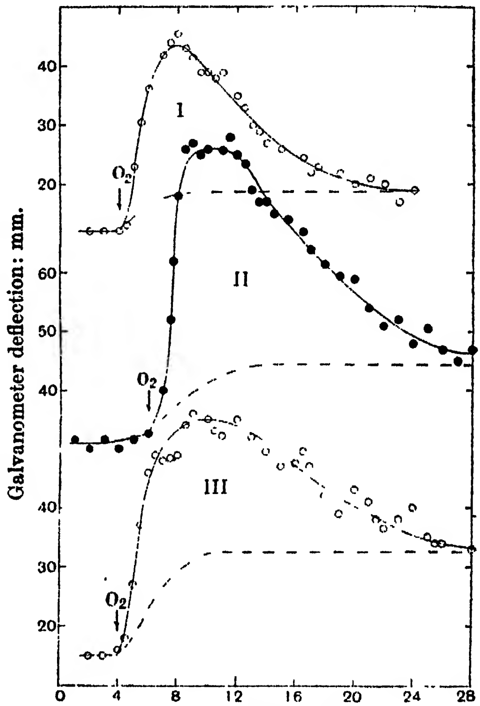

Fig. 4.

Fig. 3. Showing the relative magnitude of the stretch response in O_2 and N_2.

Fig. 4. Recovery heats: I and III after 40 min. unloaded anaerobiosis, II after anaerobiosis lasting 40 min., during 30 min. of which the muscle was loaded with 50 g. I, II and III observed in succession and all with muscles unloaded.

it should cause an extra oxygen debt which would show itself later, on admitting oxygen, in the form of extra recovery heat. All this was actually realized. Fig. 3 shows the relative magnitude of the stretch response to 20 g. loading in oxygen and in nitrogen. The former is about twice the latter, as it should be perhaps, since the usual resting heat rate in oxygen is about twice that in nitrogen. Fig. 4 shows the recovery

heat on admitting oxygen (galvanometer deflection, unanalysed) after the same period of oxygen want under different conditions. I represents the amount after oxygen want unloaded for 40 min.; II that after oxygen want for 40 min. during 30 min. of which the muscle was loaded with 50 g.; and III that after unloaded anaerobiosis again. II is obviously very much greater than I, in this particular case about twice as great: III is also somewhat greater than I, the anaerobic heat rate after stretch not having returned completely to the original level. It should be noted that the recovery heats were measured always with the muscle under the same conditions, namely unloaded, so that the difference between I and II must be due to the conditions obtaining during previous oxygen want and not to the conditions existing at the moment, which were the same in both.

It is quite clear, therefore, that stretching the muscle does increase its oxygen requirement. For further demonstration an independent method was sought and measurements made directly of the oxygen consumption of a muscle when stretched and when unstretched respectively. A Barcroft differential manometer was employed with special chambers, as shown in Fig. 5. The chambers were of approximately equal size, containing about 14 c.c. A glass rod, bent somewhat to one side, and provided with two glass hooks, was sealed to the stopper *A*. The apparatus was particularly designed for the double sartorius preparation. The small portion of pelvic bone joining the two muscles rested against the lower hook, while the free ends of the muscles were slung over the upper hook and tied, by means of thread, on to a rod projecting from a bottom stopper. Turning the latter winds up the thread and stretches the muscle: release is simply effected by turning the stopcock in the opposite direction. In this way stretch and release can be carried out without opening the chamber and repeated as often as desired. During an experiment about 0·3 c.c. of 0·7 p.c. KOH, having approximately the same vapour pressure as the muscle, was placed in the bottom

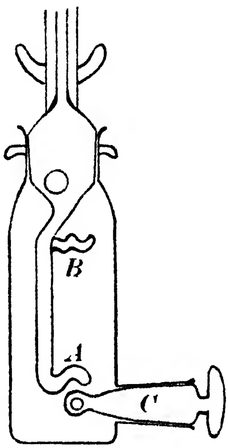

Fig. 5. O_2 consumption chamber, approximately ⅗ natural size. The small piece of pelvic bone joining the two sartorii of a double sartorius preparation rests against *A*; the muscles are slung over *B* in opposite directions and are tied on to the projecting end of the stopcock *C*. Stretching and releasing are carried out without opening the chamber, simply by turning *C*.

of the chamber and served to absorb CO_2 as well as to keep the chamber moist. The chamber containing the muscles was always filled with oxygen, the compensation chamber holding about 0·6 c.c. of KOH solution and air. The chambers were immersed in a water bath at room temperature and shaken by a to-and-fro movement about once per second. The manometer was read every 10 min., both sides being recorded and the average taken.

Some results are shown in Fig. 6. Oxygen consumption in any interval

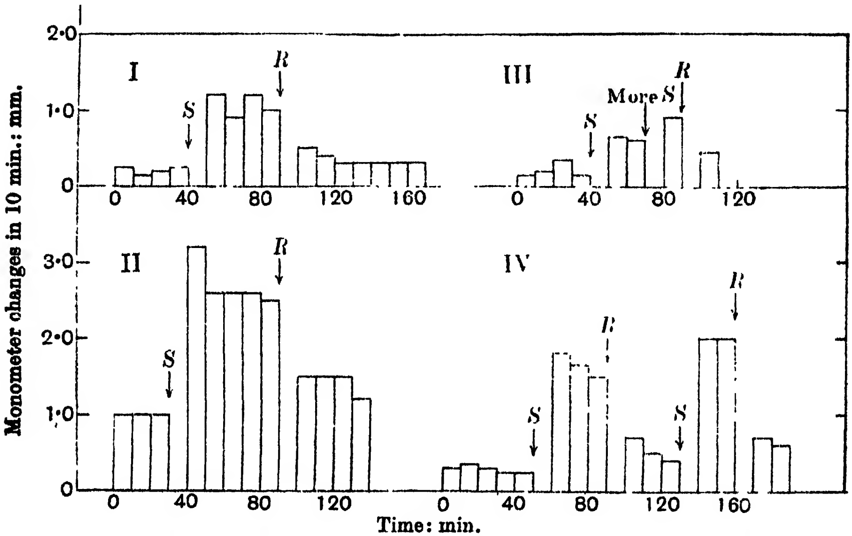

Fig. 6. Sartorius of *R. temporaria* at room temperature, showing the effect of stretch on the O_2 consumption. I, II, III, IV are four individual experiments. S = stretch, R = release. O_2 consumption expressed in mm. displacement of meniscus, each block representing 10 min.

is expressed simply in mm. displacement of the meniscus of the manometer, no calibration being made. The amount of stretch applied was not accurately known but was of the order of that due to 30 to 60 g. hung on a pair of sartorii weighing about 160 mg. As shown, stretching the muscle increases its oxygen consumption two to fourfold.

The next step in the effort to understand the stretch response was to see what change in the condition of the muscle itself could influence it. As mentioned already, the response was abolished altogether by electrocution or by chloroform, but still existed when the muscle was rendered inexcitable by soaking in high-KCl Ringer's fluid or in sugar solution. Clearly the mere ability of the muscle to contract when stimulated is not

a necessary condition of the stretch response. Its disappearance after electrocution and chloroform may of course be correctly stated as due to the muscle being dead. More probably, however, it may be attributed to the muscle being completely exhausted. The question then arises whether partial exhaustion will partially abolish it. Experiment showed this to be the case. Fig. 7 is one experiment on a muscle in nitrogen, showing how the stretch response is much diminished with each series of 50 twitches. On allowing the muscle to recover in oxygen the stretch

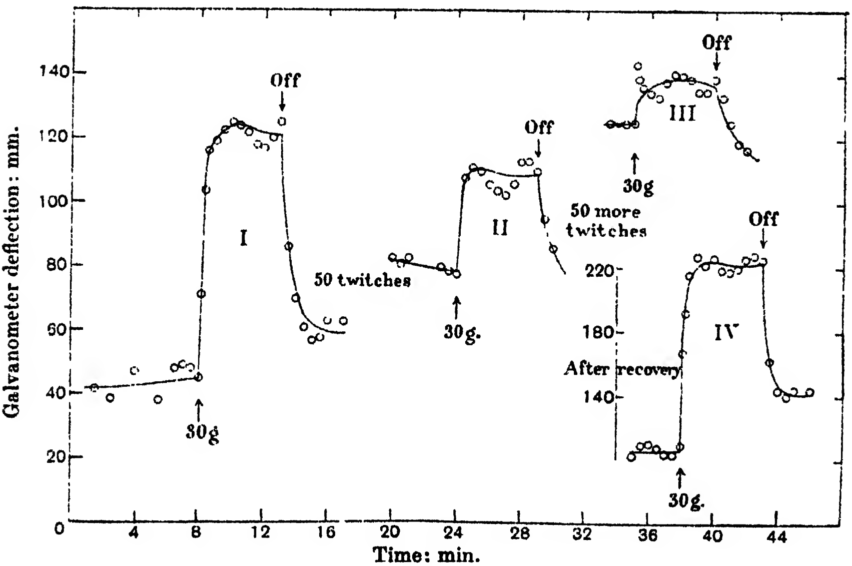

Fig. 7. Sartorius of *R. temporaria* in N_2, showing the effect of previous activity on the size of the stretch response to a load of 30 g. I, fresh muscle; II, after 50 twitches; III, after another 50 twitches; IV, after $2\frac{3}{4}$ hours' oxidative recovery. Note that the scale of the ordinates for IV is half as large as for the others.

response also recovers; often it appeared larger after oxidative recovery following stimulation than at the beginning, as in the case shown.

Muscles poisoned with iodoacetic acid give a full-sized stretch response. The effect, however, of stimulation is more pronounced, and once diminished by stimulation oxidative recovery of the response does not occur (see Fig. 8).

Ringer's fluid containing 120 mg. of extra KCl per 100 c.c. appears to make the muscle give a considerably larger stretch response to a given load. Soaking in isotonic KCl solution, however, even for 5 min., seems to diminish it, certainly not to increase it. Ringer's fluid containing

300 mg. of extra $CaCl_2$ per 100 c.c., and pure isotonic $CaCl_2$, leave the stretch response unaltered. Pure CO_2 definitely diminishes it.

The experiments reported so far were all made on *R. temporaria*. When similar experiments were tried on the large Hungarian *R. esculenta* I was surprised to find generally no stretch response at all, occasionally a small and doubtful one. The results obtained before August with this species of frog were as consistently negative as with *R. temporaria* they were positive. There was no prolonged heating effect when the sartorius

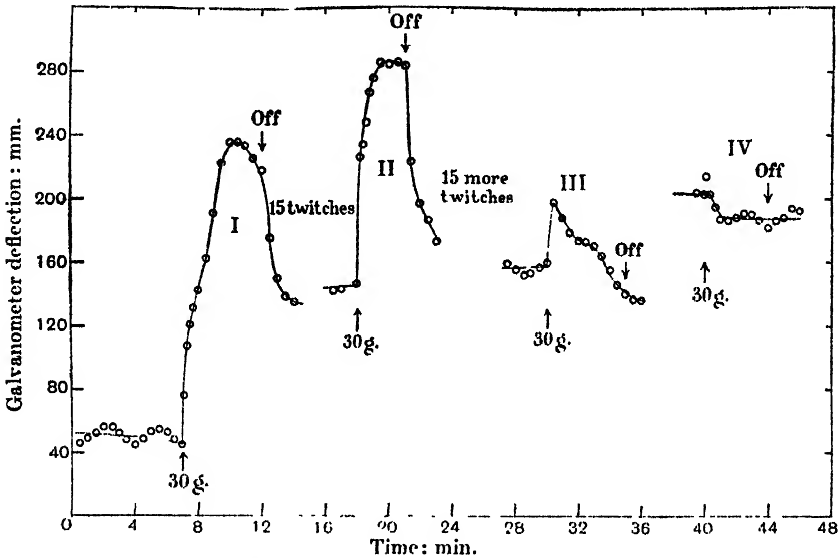

Fig. 8. Sartorius of *R. temporaria* poisoned by iodoacetic acid, in N_2. The effect of previous activity on the response is similar to that in normal muscles but more pronounced: I, before stimulation; II, after 15 twitches; III, after another 15 twitches; IV, after 45 min. in O_2; in contrast with normal muscle no oxidative recovery takes place.

of a Hungarian frog was stretched, even by a relatively much greater amount than was usually applied to the English ones. The recovery heats, moreover, after a given period of oxygen want were the same whether the muscle was stretched or not. The oxygen consumption of the muscle also was unaffected by stretching. This specific difference was most unexpected and puzzling. One would naturally be inclined to suppose that the particular batch of Hungarian frogs, at the time they were used, were in bad condition, or more precisely, in a partially exhausted condition, since, as shown above, exhaustion is so effective in diminishing or abolishing the stretch response. Actually they were not in the best

of condition as they had been in the laboratory two and a half months, but still their muscles contracted strongly when stimulated. Later, in September, a new batch of Hungarian frogs was obtained. These were certainly in excellent condition, and nine experiments were made on their sartorii. In two cases I found definite evidence of the stretch response; in two others none at all, while in the remaining five the response appeared

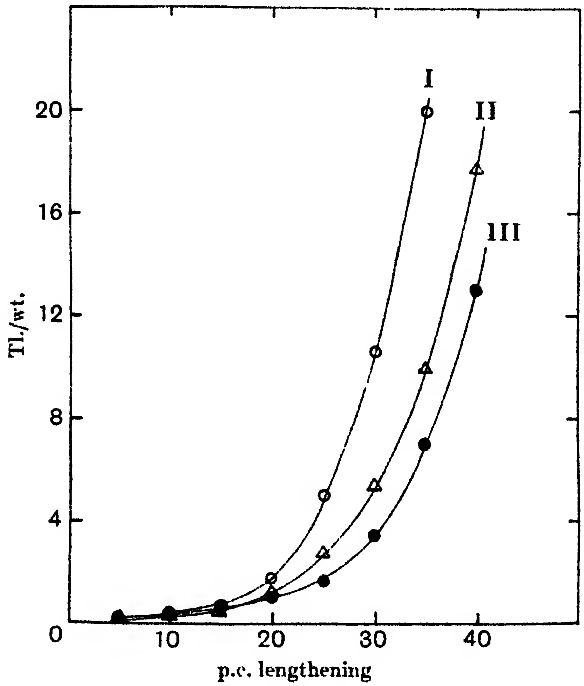

Fig. 9. Average tension-length curves of resting sartorii. I, Hungarian *R. esculenta* (average of 5 curves); II, Dutch *esculenta* (average of 4 curves); and III, English *temporaria* (average of 4 curves). Ordinate, Tl./wt., which is roughly proportional to the tension in g. per sq. mm.; abscissa, percentage lengthening reckoned from length at zero tension.

to be present but small and doubtful. After these new experiments, in which there was no question about the condition of the animals, I feel compelled to conclude that the muscles of Hungarian *R. esculenta* generally do not show, or show only to a very slight degree, the stretch response so conspicuously manifested by *R. temporaria.*

A few experiments also were made late in September on Dutch *R. esculenta* which were not available in the months before August. The

sartorii of this species in the half-dozen cases examined showed the stretch response indeed but to a considerably less extent than those of *R. temporaria*. Two experiments on the biceps cruris of the tortoise and one on the rectus abdominis of the mouse at 20° C. gave negative results.

Large specific differences therefore exist. What can be their cause? In handling the various kinds of muscles one gains a general impression that those of Hungarian frogs are considerably and those of Dutch frogs appreciably less extensible than those of English ones. This is borne out by experiment. Fig. 9 shows average tension-length curves of resting sartorii of the three species of frogs. For the better comparison of muscles of different weights and lengths, the tension per unit of cross-section (actually Tl./wt.) is plotted against the percentage lengthening. The curve for Hungarian sartorii is steepest, that for Dutch comes next, while that for English is the lowest. It is not unreasonable to suppose that the stretch response would be more prominent in more extensible muscles, and that this might be the basis of the specific difference in question. Against this, however, it may be urged that a load of 20 g. caused a conspicuous stretch response in *R. temporaria*, while 150 g. failed to evoke any response at all in Hungarian *R. esculenta*. Such differences must presumably be based upon some factor in addition to extensibility.

Discussion.

That stretching a muscle may increase its metabolism has been suspected from time to time ever since Weber first systematically investigated the extensibility of muscle. Suggestive evidence of various kinds appeared in the literature at intervals. Meyerstein and Thiry [1863] found that when a muscle was extended by a relatively large weight there was at first a slight cooling, generally followed by some heating, and they concluded that muscle, at the moment of being stretched, offered an active though weak resistance; once stretched, the muscle was thought to carry the weight passively. Schmulewitch [1867] observed a larger heat production on extending a living muscle than a dead one. Westermann [1868], however, found that thermal effects of stretch and release were the same in living and in dead muscles. A rise of temperature in a muscle when it was stretched was noticed by a number of other workers, but there is no point in enumerating all the instances here. Most probably these older observations showed only the thermo-elastic and frictional heat later studied by Hill and Hartree [1920] and gave no definite evidence of any active heat production due to

stretch. Gotschlich in 1894 (quoted by Eddy and Downs, 1921 *b*) found a production of lactic acid by a muscle when loaded.

More recently Eddy and Downs [1921 *b*], using Tashiro's barium hydroxide technique, showed that a stretched muscle liberates about four times as much CO_2 in a given time as an unstretched one. These authors used gastrocnemii and weights of 50 g. to stretch them. The fact that gastrocnemii have obliquely arranged fibres and are so rich in strong inextensible connective tissue that a weight of 50 g. will hardly extend the muscle fibres at all, makes their result, if it be correct, very striking. It is possible that American frogs greatly surpass even English *R. temporaria* in the prominence of the stretch response. More probably, however, the method employed showed a much greater difference in CO_2 output between stretched and unstretched muscles than really existed. If Gotschlich be right, an unknown fraction of the CO_2 output by stretched muscles must be due to preformed CO_2 driven out by lactic acid.

In another paper Eddy and Downs [1921 *a*] reported that gastrocnemii, previously loaded by 100 g. for periods from 30 min. to 3 hours, were fatigued by stimulation in less time than unstretched muscles. The average time to complete fatigue for unstretched muscles is 19·25 min. and that for stretched ones 16·53 min. Their results, however, showed very large individual variations: in a number of cases it took a longer time to fatigue a stretched muscle than an unstretched one. And even if the average difference be significant, it is still not necessary to conclude that the difference is due to partial depletion of available energy store in the case of stretched muscles, as the authors did.

The question will naturally be asked, how does stretch bring about an increase in the resting metabolism of a muscle? In this form the question can no more be answered than why does stimulation cause contraction and attendant energy liberation? Stretching a muscle is known [Hill, 1925] to affect the heat liberated in response to a stimulus. In this case, however, the heat increases with the stretch up to a certain quite low limit and then decreases considerably as the muscle is further stretched. With the loads used in the present experiments the heat liberated in an isometric twitch would be less and not more than that of the unloaded muscle. It is possible to discuss whether the increased length itself, or the tension causing it, is the determining factor in the stretch response. Naturally in a resting muscle tension and length vary in the same direction and there is no way to separate them. There might, however, be a simple relation between the one, or the other, and the resting metabolic rate.

In Fig. 10 is shown an experiment in which the load on the muscle was increased progressively by 10 g., and the resting heat rate following each increment observed. There is no simple proportion between heat rate and load; nor, however, it can be similarly shown, is there between heat rate and length. It is impossible in this way to decide which relation is the more fundamental.

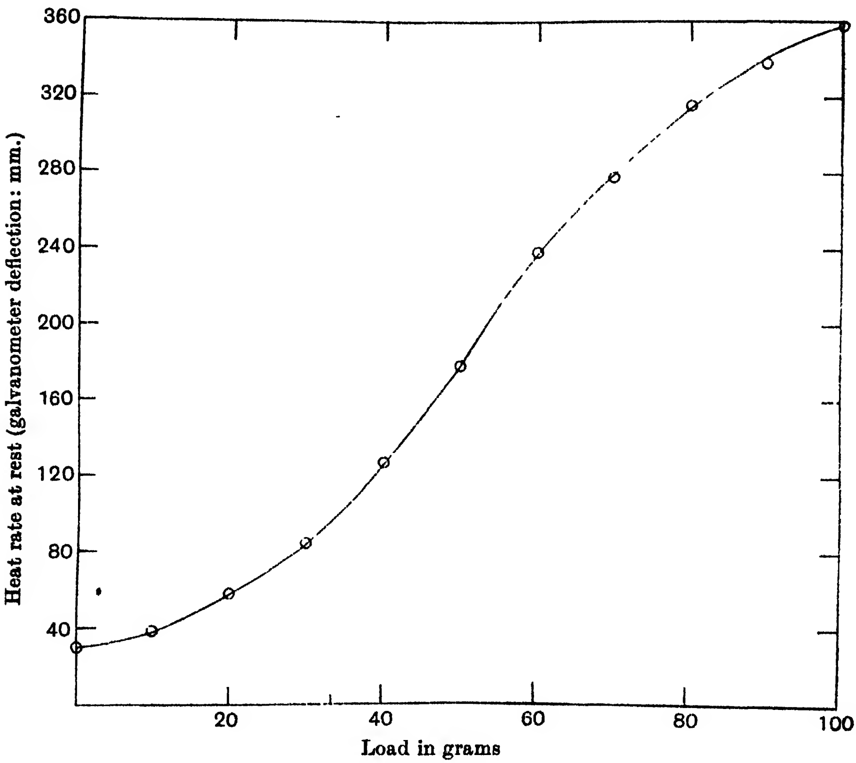

Fig. 10. The relation between resting heat rate and load. Curve starting with 2·5 g. initial load, in air.

The most striking evidence of the nature of the effect described is its intimate dependence on the previous activity of the muscle. Fifty twitches of a muscle in nitrogen often diminish the stretch response to a half or less, and this at a stage of fatigue when the ability to contract was entirely unimpaired, often indeed greater than at the beginning. If the source of energy for contraction and stretch response be one and the same, how can it be amply available for the one but nearly inaccessible

for the other? If, on the other hand, the two have distinct energy supplies, why does the performance of the one affect the other? One thing is certain: that the energy supply is not necessarily derived from the breakdown of glycogen. Soaking a muscle in Ringer's fluid containing glucose does not augment the stretch response and, as recorded earlier, it exists undiminished in muscles poisoned with iodoacetic acid. One may suspect that the energy supply concerned is related to the phosphagen complex. The high initial rate of breakdown of phosphagen under anaerobic conditions is consistent with the great diminution suffered by the stretch response as a consequence of 50 twitches. The failure of oxidative recovery of the stretch response, following stimulation in nitrogen, of muscles poisoned with iodoacetic acid, is in keeping with the absence of phosphagen resynthesis in that case. Possibly a certain high level of phosphagen concentration is requisite for the conspicuous manifestation of the stretch response, and it is tempting to suggest that the different aptitudes for this response shown by three species of frogs might have a basis in their relative contents of phosphagen, or more likely perhaps in its state of combination.

The alternative possibility that the performance of contraction might change the condition of the muscle, so as to diminish its stretch response, is not readily confirmed. An increased acidity might perhaps be suggested, particularly in view of the diminishing effect of CO_2 on the stretch response. In a muscle, however, poisoned with iodoacetic acid, stimulation causes increased alkalinity, not acidity, and according to Lipmann and Meyerhof [1930] a normal muscle, when stimulated, first becomes more alkaline, coincident with the preponderant breakdown of phosphagen, and then later more acid. No reversal of the effect of stimulation on the stretch response, when stimulation is further continued, has been found. A change of pH therefore can scarcely be the cause of the effect of stimulation.

Summary.

1. The resting metabolism of a muscle increases when it is stretched, the greater the stretch the more the increase. The phenomenon has been demonstrated, both by heat and by oxygen consumption measurements. It is referred to as the "stretch response."

2. The increment of resting heat rate due to a given stretch is about twice as great in oxygen as in nitrogen. Stretching a muscle in nitrogen causes an extra oxygen debt which can be detected in the form of a greater recovery heat when oxygen is admitted.

3. In a muscle in nitrogen the stretch response diminishes progressively with increasing amount of previous activity: 50 twitches often decrease it by one-half or more. After recovery in oxygen the stretch response is completely restored.

4. The stretch response exists undiminished in muscles poisoned with iodoacetic acid, but once diminished or abolished by stimulation in nitrogen it cannot recover in oxygen.

5. A great difference exists between the three species of frogs studied, in respect of the prominence of the stretch response. It is manifested most conspicuously by English *R. temporaria*, considerably less by Dutch *R. esculenta*, and only to a slight degree—often not at all—by Hungarian *R. esculenta*.

6. It is possible that a certain high level of the phosphagen content is requisite for a clear manifestation of the stretch response.

I wish here to express my sincere thanks to Prof. A. V. Hill for his encouragement and advice; to Mr A. C. Downing I owe my gratitude for instruction in managing his galvanometer, and to Mr J. H. Trendall for making the chambers used in the oxygen consumption measurements. To Mr J. L. Parkinson I am indebted for much assistance and advice during the course of this work.

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THE THERMO-ELASTIC PROPERTIES OF MUSCLE.

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THE thermo-elastic properties of muscle have been studied by various workers since the first attempt by Heidenhain [1863] and by Meyerstein and Thiry [1863]. Some have measured the thermal effects of stretch and release, others the actual change of length with alteration of temperature. Considerable disagreement, however, exists among both groups of investigators (see Wöhlisch and Clamann [1931] for a brief review). Hill and Hartree [1920] with the best myothermic technique then available obtained a negative thermal coefficient of linear expansion for muscle, whether living or dead. The great improvement in myothermic technique achieved in recent years suggested a re-investigation of the problem, and this is described below. During the course of it, a paper by Wöhlisch and Clamann appeared [1931] who, with the former's optical linear dilatometer, measured the variation with temperature of the length of a muscle. Their most important conclusions are: (*a*) the coefficient of linear expansion of muscle is negative when its initial extension is less than 35 p.c. of its unloaded length, and (*b*) with greater initial extension the coefficient becomes positive. Dead muscle always gave a positive coefficient. These conclusions are only in partial agreement with those of Hill and Hartree and made a repetition of their work the more necessary.

METHODS AND SOURCES OF ERROR.

The procedure first tried was similar to that adopted by Hill and Hartree. A Downing moving magnet galvanometer and a new thermopile with soldered constantan-iron junctions were the chief instruments employed. The thermopile is essentially a thin sheet of wire insulated with bakelite varnish, sometimes further coated with shellac or paraffin.

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It is considerably quicker and more sensitive than the usual silver-plated variety. Thermopiles of the latter type were used occasionally for comparison. A pair of frog's sartorii was mounted in the usual way, and connected by a wire or thread to one end of an isotonic lever, whose other end was provided with a hook on which could be hung a suitable weight for stretching the muscle.

It was early realized that before any real conclusion could be drawn a number of complications had to be cleared up. It was found in the very first experiments that muscles from different species of frog apparently gave different effects and, what was more serious, the results given by the same muscle on different thermopiles did not agree. Such common errors as temperature inequality, creeping of galvanometer zero, and electrical leaks in the thermopile, were easily excluded and need not be considered. Various puzzling difficulties were finally traced to their sources and will be briefly described. They were of two types, (*a*) physiological and (*b*) instrumental.

(*a*) *Physiological complications.* Stretching a muscle, though not provoking contraction under usual conditions, is not without a physiological consequence. In the case particularly of the sartorius of *R. temporaria* stretching causes a conspicuous rise in the resting heat production. This effect is described in detail in the preceding paper and will be referred to as the "stretch response." Fig. 1 shows how the thermo-elastic effect may be complicated by the "stretch response." A single sartorius of *R. temporaria* was subjected to various initial loads (1·0, 6·0, 16 and 31 g. respectively for curves I, II, III and IV), and then a stretch of about 2 mm. was applied, followed by a release. At 1 g. initial load 2 mm. additional stretch did not cause an appreciable "stretch response," and the effects of stretch and release were simple heating and cooling as shown, the galvanometer returning in each case to its initial base line. (Note that in all figures heating causes a downward deflection.) With 6·0 g. initial load a stretch of 2 mm. caused an obvious "stretch response," as shown by the steady heating deflection continuing thereafter. On release a small thermo-elastic heating effect is nearly masked by the cooling due to the disappearance of the "stretch response." With 16 g. initial load the "stretch response" was most pronounced, masking almost completely the thermo-elastic cooling effect of stretch (see below for the change of sign of thermo-elastic effect with increased initial load). The thermo-elastic warming on release was now much larger with the greater initial load and remained clearly visible, but would have been still more prominent had it not been partially cancelled by the cooling

due to the disappearance of the "stretch response." Curve IV for 31 g. initial load is similar to curve III and needs no further comment. Knowing that both are present and using relatively quick instruments, one can generally separate qualitatively the thermo-elastic effect from the "stretch response." At the beginning, however. of the investigation the complication introduced by the "stretch response" was not expected, and caused apparently discordant results from muscles of different species of frogs possessing different aptitudes for showing the "stretch response."

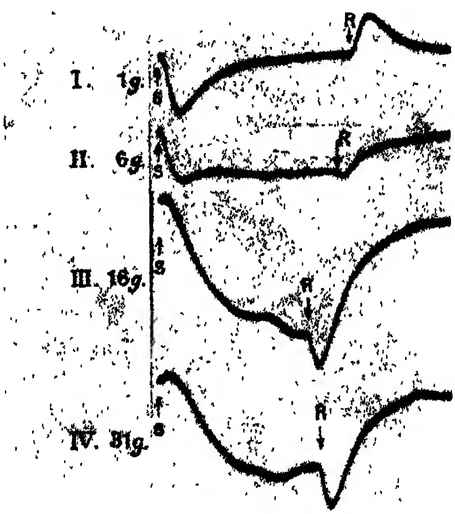

Fig. 1. Showing complication of thermo-elastic effects by "stretch response" at various initial loads. Downward deflection always heating. S = stretch, R = release in all figures. Time marks (gaps in curves) about 1·5 sec. For details see text.

(*b*) *Instrumental complications.* Since thermo-elastic phenomena occur in all elastic bodies (except those with a zero coefficient of thermal expansion) it is important in investigating the thermo-elastic effects in muscle to guard against their intrusion elsewhere, owing to the stress applied to the muscle being transmitted to part of the recording apparatus. The magnitude of this complication had not been realized, though fortunately Hill and Hartree [1920] had avoided it by allowing the stress applied to their muscles to be taken by a clamp attached not to the thermopile itself but to the chamber containing it. The thermopiles at present employed are of two types: one consisting of constantan wire

wound around a hollow frame and silver-plated, the other of a thin solid sheet of soldered constantan-iron junctions. Both are rigidly held in a brass frame in which the cold junctions are embedded and to the base of which the pelvic end of a sartorius preparation is secured by means of a screw and a clamp. The other end of the muscle is connected to a thread or wire led to the outside through a central tube above the frame. With this arrangement, when stress is applied to the muscle, three possible things may happen: (1) the force may be transmitted *via* the muscle clamp and the brass frame to the material in the neighbourhood of the "cold" junctions of the thermopile, possibly to the cold junctions themselves, and consequently thermal changes of thermo-elastic origin may occur at the cold junctions and cause a deflection of the galvanometer; (2) when the contact of the muscle with the thermopile is not exactly tangential, that is when its ends are somewhat bent inward at the upper

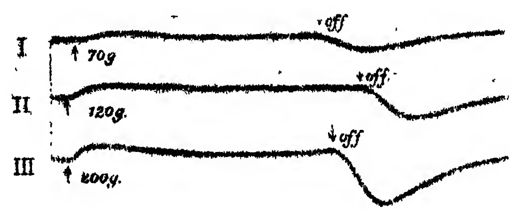

Fig. 2. Thermo-elastic effects of loading and unloading the brass frame of a thermopile, nothing being in contact with the hot junctions. I, 70 g. load; II, 120 g.; III, 200 g. Time marks about half-sec., as in all later figures unless otherwise specified.

and lower edges of the thermopile, a bending stress will be applied to the thermopile itself when the muscle is stretched and may introduce undesirable thermal effects—thermo-elastic phenomena in the "hot" junctions. This complication may occur particularly when solid thermopiles are used, which are considerably weaker than the hollow ones supported on a fairly strong silver frame; (3) in the case of the hollow thermopiles air is hermetically enclosed in the interior. If a pressure is quickly exerted on the surface of the thermopile the air inside may be momentarily slightly compressed and so its temperature caused to rise. On removing the pressure a corresponding fall of temperature would take place.

Of these complications (1) and (2) were actually encountered. Fig. 2 illustrates the effect of pulling on the brass frame of a thermopile by 70, 120, and 200 g. respectively, nothing being in direct contact with the hot junctions of the thermopile surface. Such disturbances are very

serious since their magnitude is about the same as that of the actual changes sometimes to be studied in the muscle. Fig. 3 contrasts the effect of stretching a piece of connective tissue isolated from the gastrocnemius muscle of a large Hungarian frog (*a*) when mounted with its end somewhat bent inwards at the edges of the thermopile, and (*b*) when mounted tangentially to the thermopile surface. The extra initial kicks in (*a*), if not traced to their real source (thermo-elastic effects in the thermopile itself), might obviously lead to very false conclusions regarding the properties of connective tissue. The existence of complication (3) has not been definitely verified, but it is obviously a possibility, especially in those hollow thermopiles with relatively uneven surfaces. If and when it occurs, however, (2) must happen as well and it will be difficult to separate them.

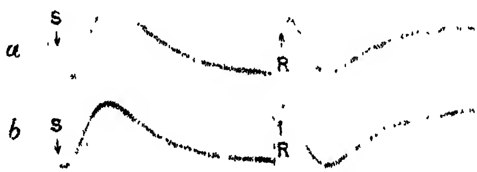

Fig. 3. Effects of stretch and release, by about 1·5 mm., of a piece of connective tissue isolated from the gastrocnemius muscle of a large Hungarian frog: (*a*) mounted with its ends somewhat bent inwards at the edges of the thermopile so that when pulled it exerted a bending stress on the hot junctions; and (*b*) mounted tangentially to the thermopile surface. Note the extra initial kicks in (*a*).

The experience thus gained led finally to the adoption of the following procedure. Most experiments were made on the sartorius of Dutch *R. esculenta* which is of suitable size for the thermopiles in use, and in which the "stretch response" is generally not very conspicuous. All the observations, except those in a few later quantitative experiments requiring calibration, were made on muscles immersed in Ringer's solution continuously well-stirred by a stream of bubbles of nitrogen: this rules out with certainty the possibility of temperature inequalities along the muscle. It has the further advantage that the deflection due to the resting heat production is made negligible, so that slight changes in the amount of muscle in contact with the thermopile, brought about by stretch and release, have no perceptible effect. Solid[1] thermopiles only were used, partly to exclude the perhaps remote possibility of adiabatic

[1] That is, not of the hollow type: actually the wires form a wafer-like layer, only a few tenths of a millimetre thick.

compression of the air in the interior of the hollow ones, but chiefly to obtain quickness and sensitivity, since working with stirred Ringer's fluid, the rate of heat loss is extremely great. Since it is not easy to ensure tangential contact of each muscle with the thermopile when there is one muscle on each side, a single sartorius was employed on one surface only.

The most important point is the manner of fixing the muscle in the chamber. The pelvic bone of a single sartorius preparation was held in an ordinary muscle clamp a little below the thermopile and slightly away from the thermopile surface. The portion of muscle opposite the thermopile was gently kept in by a piece of silver wire to give a just good contact. The clamp, instead of being provided with a short rod fixed by a screw to the brass frame, as is the usual practice, was provided with a long rod loosely going through the hole in the base of the thermopile frame, and projecting through an opening in the bottom of the glass chamber. The projecting end was threaded and a nut served to fix it against the bottom of the combined muscle and thermopile chamber. With this arrangement the stress applied to the muscle can be transmitted only to the glass chamber, which of course does not matter.

In stretching and releasing a muscle there are obviously three experimental variables to be considered: (1) the amount of stretch, (2) the rate of stretch, and (3) the initial length of the muscle from which the stretch starts. All three influence the results observed. To have them under control the simple procedure of stretching the muscle by a weight is not permissible. Stretching and releasing therefore were carried out by means of a Levin-Wyman ergometer [1927] which allows accurate control of the first two variables. The third was varied by changing the constant load applied by an isotonic lever to the muscle. Photographic records of the galvanometer deflection were taken on bromide paper.

Results.

The thermal effects of stretching a muscle are by no means constant. With different combinations of the three variables involved, *i.e.* initial load, and amount and speed of stretch, most diverse results could be obtained. The most important factor is the initial load. With a small initial load stretching has a warming, and releasing has a cooling effect; increasing the initial load completely reverses the phenomena (see Fig. 4). The thermo-elastic effect at 22 g. initial load is exactly the opposite of that at 2·0 g. By increasing the initial load in small steps the thermo-

elastic effect can be observed to pass gradually from one type to the other. Fig. 5*a* shows such an experiment at 20° C., and Fig. 6 a similar one at 0° C.

This reversal of thermo-elastic effect by increasing the initial load can be shown in another way on a single curve. Starting with a small initial load, let us give the muscle a large amount of stretch. The later part of the stretch will then in effect be a stretch at a large initial load. The deflection curve for a large stretch will therefore contain a later cooling phase succeeding an initial warming one. The actual shape of the curve obtained depends upon the speed at which the stretch is carried out. At high speeds the warming and cooling will be summed together and only their algebraic sum will be shown, complicated, of course, by a large irreversible (viscous) heating. On the other hand, at a low speed the two phases will be spread out, and the warming followed by a cooling will be clearly exhibited on a single curve.

Fig. 4. The reversal of thermo-elastic effect with increase of initial load. I, at 2 g. initial load, stretch and release cause heating and cooling respectively. II, at 22 g. the effects are exactly the opposite.

Fig. 7*a* shows three pairs of curves taken in order. I are stretch and release curves at a very low speed; II are the same at a high speed; III are at the low speed again; the amount of stretch and the initial load being the same for all, 6 mm. and 2 g. respectively. The difference between I and III on the one hand, and II on the other, is very striking. It is easily intelligible, however, in the light of what was said above. The amount of stretch employed was large enough to cause the thermo-elastic property of the muscle to change its sign during the course of the stretch. The slow stretch curves accordingly show a warming phase succeeded by a cooling, which in this particular instance is more pronounced. The slow release curves show first a large warming phase and then a cooling just visible. They may be roughly described as stretch curves turned upside down and also right side left. The quick stretch curve shows an initial cooling which may roughly be regarded as the algebraic sum of the phases separately shown on the slow curves. The later heating is due to the large amount of energy irreversibly dissipated by internal friction in a muscle when rapidly stretched. When the initial

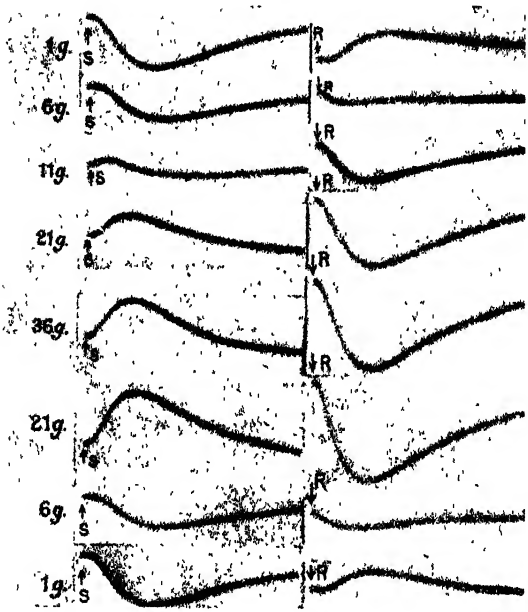

Fig. 5*a*. At 20° C. Showing the gradual reversal of thermo-elastic effect when the initial load is increased in small steps: the amount and speed of stretch being kept approximately the same.

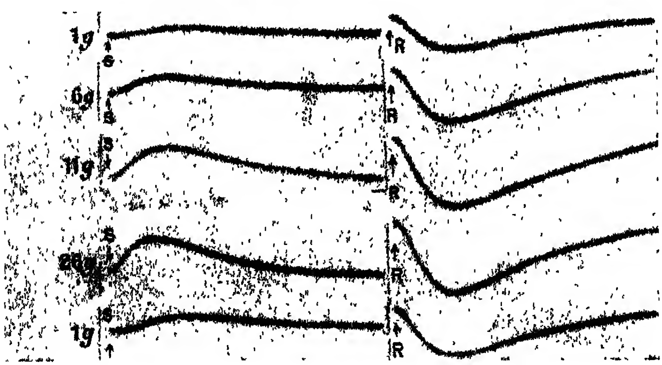

Fig. 5*b*. Repetition of observations shown in Fig. 5*a* on the same muscle, under precisely the same conditions, on the following day when it had become inexcitable. **Note that the warming effect on stretch and cooling on release at small initial loads are no longer obtainable.**

warming phase is relatively more prominent the quick-stretch curve takes a simpler appearance (Fig 7*b*).

It is clear, therefore, that the thermo-elastic properties of a muscle depend on the initial load. The load required to reverse the sign of the thermo-elastic effect varies somewhat from muscle to muscle, and, since the transition is a gradual one, it is difficult to fix a definite point. Generally speaking, an initial load of 20 g., applied to a single sartorius of Dutch *R. esculenta*, weighing about 100 mg., is sufficient to cause a complete reversal. This reversal can also be readily observed in the sartorii of English *R. temporaria* and Hungarian *R. esculenta*. Occasionally, but rarely, cases were met in which, even at the minimum load of 1 g., stretch

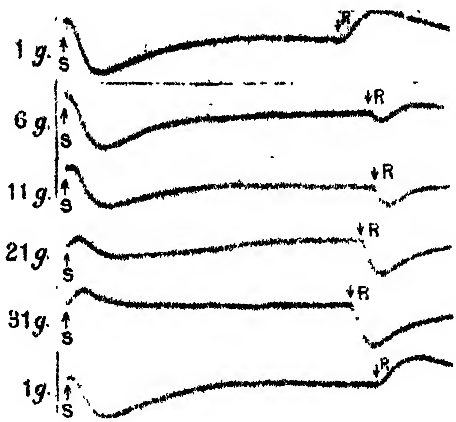

Fig. 6. At 0 C., otherwise similar to Fig. 5*a*. Time marks about 1 sec.

already gave rise to a cooling and release to a warming. Even in such cases, however, increasing the initial load made the cooling and the heating more conspicuous for a given amount of stretch and release.

Dead muscles, or, more precisely, muscles which have been partially fatigued and left in nitrogen or oxygen-free Ringer's fluid overnight and have lost their excitability, only give thermo-elastic effects corresponding to a positive coefficient of expansion. The muscles, however, in the first four or five cases encountered, were all in a somewhat rigid and extended condition. To test whether the change in their thermo-elastic properties was secondary to a change in length, or to a true alteration resulting from death, the following experiment was made. After having first ascertained that with small initial load the muscle gave the usual warming on stretch and cooling on release, it was stimulated to partial exhaustion

and left on the thermopile in Ringer's fluid with nitrogen bubbling, and with all its connections to the lever and the Levin-Wyman ergometer unchanged, a note of its length as indicated by a pointer being kept. It was tried again next morning with everything as on the preceding day. Its length was first noted and showed no appreciable alteration. Only thermo-elastic effects corresponding to a positive coefficient could now be observed (Fig. 5*b*). This suggested some real change in thermo-elastic properties as the result of death, other than connected with a change of

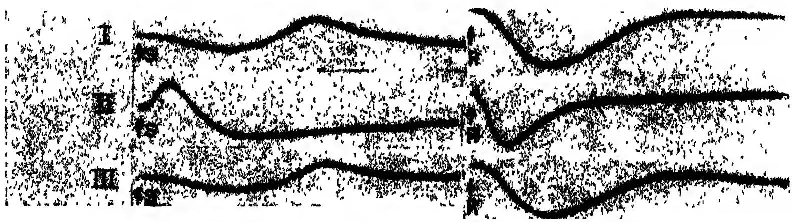

Fig. 7*a*. I, II and III are pairs of curves taken in succession, to contrast the apparent thermal effect of a large stretch or release (6 mm.), (*a*) when carried out slowly (I and III), and (*b*) when carried out quickly (II). See text for explanation.

Fig. 7*b*. I, quick-stretch and release curves; II, slow ones: the initial load and the amount of stretch being the same, 2 g. and 6 mm. respectively. The quick-stretch curve is simpler in appearance than that shown in Fig. 7*a*, owing to the warming phase being relatively more prominent (see text).

length. It is important, however, to note that all such experiments had been made at 20·4° C. When they were repeated at 0° C. it was at first somewhat surprising to find that muscles two to three days after complete loss of excitability showed no change in their thermo-elastic properties. When such muscles were transferred to 20·4° C. they at first gave the same thermo-elastic effect as they had previously given at 0° C. In about 2 hours, however, definite changes took place, warming on stretch and cooling on release being now no longer observable. The reason appears to be this: mere loss of excitability does not alter the thermo-elastic properties of muscle, at least not qualitatively. For a change to take

place certain secondary processes, probably coagulation or other colloidal alteration, must occur which are observed to lead to a turbid and whitish appearance in the muscle. Such processes being slow at 0° C., a muscle at that temperature retains its normal appearance for several days after complete loss of excitability and so keeps its thermo-elastic properties unchanged. At 20·4° C. the rapid colloidal change brings about turbidity in a few hours, and with it an abolition of the thermo-elastic effect corresponding to a negative coefficient of thermal expansion.

Further evidence on this point was obtained with muscles in a condition of extreme rigor, their length being hardly more than one-half of normal. Though such muscles are more like a plastic mass than an elastic body, a sufficient amount of elasticity apparently remains to allow some reversible thermo-elastic effect to be obtained. When such muscles are pulled out rapidly the effect is always a large irreversible heating. If, however, they are stretched very slowly by a small amount, thus reducing the irreversible heating to a minimum, a definite cooling effect can usually be obtained. In any case releasing such muscles always gives rise to a warming effect. Similar results were given by chloroform- and heat contracture-muscles. Mere contracture, however, must not be supposed to abolish the effect corresponding to a negative coefficient: for the thermo-elastic properties of a muscle poisoned by iodoacetic acid and sent into contracture by stimulation, without changing its optical appearance, are qualitatively the same as those of fresh muscles. It is only after keeping, when turbidity begins to appear, that the reversal takes place.

In experiments carried out in Ringer's fluid a direct calibration of the heat in absolute units is impossible. Usually, however, the deflection given by a single muscle twitch was noted. From the known average heat production per g. of muscle due to a single twitch, a rough quantitative estimation of the thermo-elastic effect, and a calculation of the corresponding coefficient of thermal expansion (α) can be made, assuming the equation deduced by Hill and Hartree [1920]. The result is that α, whether positive or negative, lies between 10^{-4} and 10^{-5}. For confirmation of this several experiments were made in nitrogen, with direct calibration by the method of condenser discharge [Bozler, 1931; Hill, 1931].

This method is very much simpler than the old one, and if necessary can be applied, at any rate with approximate results, to a living muscle as follows: A condenser discharge of V volts and C microfarads is sent through the muscle, giving a deflection A representing the sum of $5CV^2$ ergs and the energy due to a single twitch. The capacity is then doubled

and a deflection B is now obtained. Assuming the twitch to have been unaltered by doubling the capacity

$$B - A = 5CV^2 \text{ ergs.}$$

For greater accuracy several values of A and B may be observed alternately and averaged.

It was at first thought desirable to carry out such calibrations at the beginning of each quantitative experiment, so that in case the muscle should be pulled to pieces in the middle of the experiment observations already made might be saved. Since care was generally taken to limit observations to the range within which there was no danger of breaking the muscle, this precaution was unnecessary and not very often practised.

In the equation [Hill and Hartree, 1920]

$$Q = \frac{\alpha T \text{ (increase of tension) (mean length)}}{4{\cdot}26 \times 10^4},$$

there are three variables: the increase of tension in g. weight, the mean length in cm., and Q the heat production or absorption in calories due to stretch or release. The wire connecting the muscle to the lever being provided with a pointer moving along a scale, the procedure was simply to lower a weight slowly and without any jerk on to the muscle by means of the Levin-Wyman ergometer, to read the deflection and to note the lengthening indicated by the pointer. The increase of tension is simply the weight employed. Q is calculated from the galvanometer deflection and the calibration number in the usual way, and the mean length is deduced from subsequent measurements of the muscle length corresponding to some one position of the pointer. Since the thermal effect of loading changes its sign with increase of initial load there are obviously two values of α to be determined, one negative and the other positive; and the matter of choosing both the load to be added and the initial load becomes important. They must be such that, in determining the negative α, the load added to whatever initial load was already there does not pass the reversal point: for the positive α, the initial load must be sufficiently large to have caused a complete reversal of the thermo-elastic effect. Generally 5·0 g. load and 1·0 g. initial load for the negative α, and 30 g. load and 20 g. initial load for the positive α are suitable, taking the case of a single sartorius weighing about 100 mg. Only when the deflections of stretch and release are approximately equal and opposite are the observations used in calculation, the average for stretch and release being taken. With the relatively small weights employed, and the slow speed at which the weights were lowered, the irreversible heating was not large and must be practically eliminated by averaging the effects of stretch and release. The values obtained for α are shown in the table below. The positive α appears consistently to be smaller numerically than the negative α.

No. of exp.	Temp. °C.	Increase of tension g. wt. for $-\alpha$	Increase of tension g. wt. for $+\alpha$	Initial load: g. wt. for $-\alpha$	Initial load: g. wt. for $+\alpha$	$-\alpha$	$+\alpha$
1	20·4	5	30	1·0	21	$-1{\cdot}4\times10^{-4}$	$+3{\cdot}2\times10^{-5}$
2	20·4	5	30	1·0	21	-7×10^{-5}	$+2{\cdot}9\times10^{-5}$
3	20·4	10	50	1·0	21	-7×10^{-5}	$+3{\cdot}4\times10^{-5}$
4	0	—	20	—	21	—	$+4{\cdot}0\times10^{-5}$
5	0	5	30	1·0	21	$-1{\cdot}4\times10^{-4}$	$+3{\cdot}5\times10^{-5}$

In the equation for calculating α the most influential quantity is the increase of tension: the mean length being used, the change of length has only a small effect.

Discussion.

In view of the present results the original conclusions of Hill and Hartree [1920] require restatement. Their main observation that the thermo-elastic effect in muscle corresponds to a negative coefficient of linear expansion is confirmed, but with the proviso that the initial load must be small. Since they never used an initial load greater than 5 g. for a pair of sartorii, they naturally missed the effect corresponding to a positive coefficient. Their finding that the thermo-elastic property of muscle is the same whether the muscle be alive or dead need not be taken as in conflict with that of the present writer on the same point. This is clear if it be remembered how different results may be given by so-called dead muscles at 0 and 20·4° C. The disagreement is perhaps only in the definition of "death," or rather of the stage of death at which we happened respectively to make our observations. It may now be stated that the thermo-elastic properties of dead muscle, before the onset of turbidity, are qualitatively the same as those of fresh muscle; with the coming on, however, of turbidity, presumably indicating colloidal and other disintegrative changes, the effect corresponding to a negative coefficient of linear expansion disappears.

It is notable that the present study of the thermo-elastic properties of muscle, investigating the thermal effect accompanying its dimensional change, has led to conclusions in complete agreement with those of Wöhlisch and Clamann [1931] investigating conversely the dimensional effect accompanying thermal change. While such agreement is thermodynamically necessary, this is perhaps the first time that it has been satisfactorily realized by experiment.

In a composite structure, like muscle, there are at least two ways in which high initial extension may be conceived to bring about a reversal

of the thermo-elastic effect. (A) It may be supposed that at small initial extensions only elements with a negative coefficient of thermal expansion are stretched, and other elements with a positive coefficient come in with increase of initial extension. If the effects of these latter are the larger the result will be an apparent reversal of the thermo-elastic effect by an increase of initial load. Let us consider muscle fibres and connective tissue fibres only, and assume for the former a negative coefficient throughout. The latter, according to Wöhlisch and Clamann [1931], have a positive coefficient. This is confirmed by Fig. 3 of the present paper, where the effect of stretching connective tissue isolated from a gastrocnemius is shown to be cooling. Since the connective tissue fibres in the muscle are not of the elastic variety, they must run a wavy course. At small initial loads, therefore, they will probably only be straightened out a little, not stretched, when the muscle is extended by a small amount. Any thermo-elastic effect observed will be due to muscle fibres alone, and so correspond to a negative coefficient. As the initial extension is increased the connective tissue fibres become taut and so will experience an actual stretch on further extending the muscle as a whole. The effect now observed will represent the algebraic sum of the effects due to connective tissue and to muscle fibres separately. To account for the reversal as actually found it is only necessary that the effect due to connective tissue should be greater than that due to muscle fibres. This may well be the case, since further increase of tension of an initially extended muscle is probably largely borne by its poorly extensible connective tissue components; and, as pointed out earlier, it is increase of tension that is the most influential factor determining the magnitude of the thermo-elastic effect. The change of thermo-elastic property with death is also easily intelligible on this view, since the muscle fibres are the first to disintegrate, the connective tissue being far more resistant.

The alternative way (B) of regarding the matter is simply to suppose that muscle fibres undergo a true reversal of their thermo-elastic properties under a large initial load. This reversal, the converse of that in rubber, would be from a negative to a positive coefficient. Muscle fibres then would form an exception to Engelmann's [1895] idea of correlation between double refraction and negative coefficient of expansion, for Ebner (quoted by Engelmann, 1895) found that the power of double refraction of muscle increases during extension. Even, however, if such true reversal occurred, the effect due to connective tissue components must still be greater at higher initial extension and necessarily contribute to the observed effect.

In attempting to consider the bearing of the observed thermo-elastic properties of muscle upon work on the heat produced as the result of stimulation, we are faced with grave theoretical uncertainties both in isometric and in isotonic contractions. In the former there is a large increase of tension with no change, or only a slight decrease, of length. This increase of tension, however, is brought about by physiological activity and is not functionally related to changes of length. It is doubtful whether the equations governing the thermo-elastic effects are applicable to such a case: though they would be to the connective tissue by which the stresses developed are transmitted. In isotonic contractions, on the other hand, there is a relatively large decrease of length at practically constant tension; the increase of tension being zero, the thermo-elastic effect also would be zero if the equation given above were applicable. The same difficulties exist in attempting to answer the question whether the phenomena of after-extension at constant load, or the after-diminution of tension at constant length, when the muscle is suddenly loaded or stretched, will be accompanied by what might be called secondary thermo-elastic effects.

Perhaps Azuma's experiments [1924] are susceptible to consideration from the standpoint of thermo-elastic phenomena. In his experiments the muscle was allowed to shorten 2 mm. by a quick release mechanism during various phases of contraction; or it was extended 1 mm. at various moments after the stimulus was given: in both cases the effect on the heat production was observed. He was able to obtain results showing that the effect of stretching was very nearly the exact converse of that due to release, thus suggesting a reversible nature of the phenomenon. In spite, however, of the reversible appearance of his results it is doubtful whether the increase and decrease of heat production by stretching and releasing are merely due to thermo-elastic effects superimposed upon a physiological heat liberation. It might have been expected from the present study that his curves for stretch and release would have been reversed, or at any rate very different, if a larger initial load had been used. Exactly what initial load he employed is not stated in his paper: from the length and the weight of the muscles given it may be inferred to be from 3 to 5 g. to a pair of sartorii, an initial load at which the predominant thermo-elastic effects for most muscles will be a warming on stretch and a cooling on release. This agrees with the greater portion of his curves so far as the direction of change is concerned. His experiments have not been repeated systematically at different initial loads, but there is no lack of examples in the literature showing that the effect of release at various stages of contraction depends upon the initial load, the effect with large initial load being the opposite of that with small (see *e.g.* Hill, 1930).

The whole problem of energy liberation in a muscular contraction in which the muscle is allowed, or made, to change its length is most involved. The influence of work done and the effects of length are inextricably mingled together (see Hill, 1930) and—on the top of these—thermo-elastic effects must be an added complication. Unfortunately the thermo-elastic properties of the active contracting muscle are not known (unless perhaps they are those of a muscle in contracture after iodoacetic acid poisoning), so it is impossible to estimate the magnitude of this factor, if and when it comes in. Qualitatively it may be said that shortening at small initial load will be accompanied by cooling, at large initial loads by warming, agreeing with the facts now known that an isotonic twitch liberates more energy than an isometric one at a relatively large initial load, and less at a small initial load. Even this statement, however, is subject to the doubts raised in a preceding paragraph.

Summary.

1. The reversible thermal effect of stretching a muscle is not constant, but depends upon the initial load: with a small initial load it is a warming, as previously found by Hill and Hartree: with a large initial load it is a cooling. Generally an initial load of 20 g. to a sartorius weighing about 100 mg. completely reverses the thermo-elastic effect from the former to the latter type.

2. Dead muscles showing obvious turbidity exhibit only the thermo-elastic effects given by highly loaded muscles.

3. The magnitude of the thermal coefficient of linear expansion of muscle, negative or positive, calculated from the thermo-elastic effect at small and at large initial loads respectively, is of the order of 10^{-4} to 10^{-5}.

4. It is suggested that the reversal of the thermo-elastic properties caused by initial extension is only apparent. The thermo-elastic property of the connective tissue of the muscle is shown experimentally to correspond to a positive coefficient of linear expansion. The reversal observed is simply the result of the connective tissue effect, coming in at higher initial extensions, masking that due to muscle fibres, assuming for the latter a negative coefficient throughout.

5. The results agree with those deducible from measurements by Wöhlisch and Clamann of the change of length caused by alteration of temperature.

The present work was undertaken at the suggestion of Prof. A. V. Hill, to whom I owe deep gratitude for guidance and encouragement. I wish also to thank Mr A. C. Downing and Mr J. L. Parkinson for much advice and assistance.

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THE CHANGES IN THE CO_2 PRESSURE AND HYDROGEN ION CONCENTRATION OF THE ARTERIAL BLOOD OF MAN WHICH ARE ASSOCIATED WITH HYPERPNŒA DUE TO CO_2.

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THE original experiments of Haldane and Priestley [1905] showed how small was the rise of alveolar CO_2 pressure which accompanied even a considerable degree of hyperpnœa induced in the normal human subject by breathing air containing CO_2, and gave the first indication of the extreme sensitiveness of the respiratory centre to changes in the CO_2 pressure in the arterial blood which reached it. Subsequent observations by Campbell, Douglas, Haldane and Hobson [1913] and by Campbell, Douglas and Hobson [1914] confirmed these results. By the time that these later observations were made all the existing evidence pointed to the fact that the respiratory centre responded not to changes in the pressure of CO_2 as such but to alterations in the hydrogen ion concentration of the blood resulting from the variations in CO_2 pressure. As Hasselbalch and Lundsgaard's [1912] determinations of the relationship *in vitro* of hydrogen ion concentration and CO_2 pressure in blood had become available, it was possible to infer from their published curves the changes of hydrogen ion concentration in the arterial blood leaving the lungs in the CO_2 hyperpnœa experiments. The conclusions, however probable they might seem, admittedly suffered from the defect that they depended on inference and not on direct measurement. The hydrogen electrode was unsuitable for making such direct measurements on the arterial blood, but the recent development of the glass electrode has rendered this possible, and this type of electrode has been frequently used during the last few years to ascertain the hydrogen ion concentration of blood obtained by direct arterial puncture under a variety of conditions. We therefore determined to re-examine as carefully as possible the changes of CO_2 pressure and hydrogen ion concentration in the arterial blood during a CO_2 hyperpnœa when there was no question of deficiency of oxygen coming into the picture.

This point is, after all, one of fundamental importance in the physiology of respiration. So far as the respiratory centre (using this term in its broadest sense so as to include not only the medullary centre but any peripheral end-organ, *e.g.* the carotid sinus nerve apparatus, which may be connected with it) is sensitive to chemical stimulation, its variations in activity will presumably depend, as has been so often emphasized by Gesell [1925], on chemical changes taking place within the constituent nerve cells themselves. Measurement of the CO_2 pressure and hydrogen ion concentration within the cells of the respiratory centre may be beyond our powers at present, but what we can ascertain is the degree to which the respiratory centre is influenced by chemical changes in the blood which reaches it. As chemical changes in the blood are ordinarily determined by variations in the activity of the different tissues of the body, we can by this means obtain an insight into the accuracy with which the activity of the respiratory centre is correlated with the metabolism of the body as a whole.

While many facts suggest that it is to changes of hydrogen ion concentration rather than to changes of actual CO_2 pressure that the respiratory centre is susceptible, there have not been wanting those who maintain the opposite view—the conclusions of Heymans, Bouckaert and Dautrebande [1930] may be cited as a recent instance. Yet many of the observations which at first appear contradictory can be brought into line with the hydrogen ion theory if attention is paid to the work of Jacobs [1920, 1922], for he has shown that whereas living cells may be readily permeable to CO_2 or H_2CO_3 in solution, many ions, including apparently even the hydrogen ion, may only penetrate with great difficulty, so that when a cell is exposed to changes of hydrogen ion concentration in its liquid environment intracellular changes of hydrogen ion concentration are far more readily and quickly caused if that environment contains free CO_2 in solution than if its hydrogen ion concentration is determined by factors other than CO_2. Hydrogen ion concentration and CO_2 concentration are necessarily interdependent, and what we are in the main concerned with in the present investigation is the precise value of the change in each case which is associated with a given alteration in the magnitude of the breathing. Jacobs's work suggests, however, that if we hope to gauge the true changes of hydrogen ion concentration correlated with changes in the respiratory centre's activity from changes in the hydrogen ion concentration in the arterial blood we should be well advised to bring about alterations in the hydrogen ion concentration in the blood merely by varying the CO_2 concentration

rather than by other means. Both direct evidence given by analysis of the CO_2 content of the arterial blood and indirect evidence afforded by a study of the rate of diffusion of CO_2 through the pulmonary epithelium have made it certain that under normal conditions the CO_2 pressure in the arterial blood is sensibly equal to that in the alveolar air, and the alveolar CO_2 pressure may therefore be regarded as a correct index of arterial CO_2 pressure.

The experiments to be described were all made on Douglas, who had served as the subject in many of the earlier experiments. Havard was well acquainted with the technique of the glass electrode and had had much practice in performing arterial puncture. The latter point was important. It was essential that the puncture should be done quickly and certainly so as to avoid disturbing the subject, since that might have led to a sudden alteration in the breathing at the critical moment which would have proved fatal to the success of the experiment. Puncture of the radial artery is apt to be painful, and pain may cause the subject to gasp or to hold his breath. Luckily we found that Douglas's brachial artery could be punctured just above its bifurcation close to the inner side of the biceps tendon without causing any pain at all, and we believe that the arterial puncture in his case left his breathing completely undisturbed. All experiments were made in the morning with the subject in the post-absorptive state, *i.e.* having had no food since the evening before: in this way we hoped to avoid variations in alveolar CO_2 pressure and in the hydrogen ion concentration of the blood dependent on the secretion of the digestive juices. The subject remained at rest throughout the experiments, reclining in a deck chair.

The experiments in which air containing CO_2 was breathed were made in an air-tight chamber of about 250 c. ft. or 7000 litres capacity. We felt it advisable not to set the concentration of CO_2 too high lest excessive hyperpnœa should hamper the observer when doing arterial puncture, while it seemed likely that only inconvenience would result if the observer tried to escape the difficulty by breathing fresh air through valves. We decided therefore not to exceed a concentration of CO_2 of $5\frac{1}{2}$ p.c. The CO_2 cylinder was outside the chamber and $12\frac{1}{2}$ to 13 c. ft. of the gas were run into the chamber through a gas meter in the different experiments. Dr J. G. Priestley was kind enough to do this for us. As soon as all the CO_2 had been admitted a 15-in. propeller fan was started in the chamber so as to ensure thorough mixture of the atmosphere, and this fan was kept running continuously throughout the experiment. In order to compensate for the CO_2 given off by the two persons in the

chamber, and thus to keep the CO_2 concentration constant, a steady ventilating current of about 0·5 c. ft. of fresh air per minute was drawn through the chamber by a centrifugal fan fitted with a suitably choked inlet. This constancy was realized in three of the experiments, but on June 10 the CO_2 percentage increased throughout the experiment for an accidental reason which will be mentioned later. No precaution was taken to keep the oxygen percentage constant, and it therefore fell slowly during the course of the experiment owing to the consumption of oxygen by the occupants of the chamber. Owing to the diminution of the difference between the inspired and the alveolar air in consequence of the hyperpnœa, the alveolar oxygen percentage rose above the normal value shown when breathing pure air in spite of the fall in oxygen concentration in the chamber.

In order to diminish the chance of experimental error in taking the samples of alveolar air, we adopted the procedure used by Campbell, Douglas and Hobson. Four equal small samples from the last portion of four different deep expirations given in the usual Haldane-Priestley way were taken into the same gas sampling tube by allowing equal amounts of mercury, with which the tube had previously been filled, to run out. Separate composite samples were obtained from deep expirations given at the end of a normal inspiration and of a normal expiration. In practice the deep expirations were given alternately at the end of a normal inspiration and of a normal expiration. An interval of about a minute was allowed to elapse between the deep expirations, the whole procedure taking from 6 to 8 min. The CO_2 percentage in the expiratory sample was on the average 0·16 higher than in the inspiratory sample when breathing pure air and 0·10 higher when breathing air containing CO_2. The figures given in Table I are the average of both inspiratory and expiratory samples, and each therefore represents the result of eight individual deep expirations, four given at the end of normal inspiration and four at the end of normal expiration.

The course of a complete experiment was as follows. Observations were first made in a well-ventilated room with the subject breathing normal air. After half an hour's preliminary rest alveolar air samples were collected in the manner just described. The respiratory exchange and total ventilation of the lungs were then determined by the bag method, the subject breathing through the valves to air for 5 min. before the expired air was switched into the bag. About 10 min. were required to fill the bag, and meanwhile a sample of the air in the room was taken in order to check its composition. Finally the observer washed his hands,

TABLE I.

	Inspired air		Respiratory exchange per minute			Alveolar air						At 37°, moist and prevailing barometer		pH of arterial blood	
Time	CO_2 p.c.	O_2 p.c.	c.c. CO_2 at S.T.P.	c.c. O_2 at S.T.P.	R.Q.	CO_2 p.c.	O_2 p.c.	R.Q.	CO_2 mm. Hg.	O_2 mm. Hg.	Breaths per minute	Litres expired air per minute	c.c. per breath	Inferred from pH CO_2 curve *in vitro*	Determined by glass electrode
March 26	0·07	20·89	195	250	0·78	5·50	13·25	0·66	39·7	95·5	11·2	7·2	643	7·375	7·416
12·25	5·35	19·55	—	—	—	—	—	—	—	—	—	—	—	—	—
12·49	5·23	19·40	—	—	—	6·14	18·07	0·61	44·3	130·1	—	—	—	7·340	
12·59	5·27	19·23	197	247	0·80	—	—	—	—	—	19·1	33·1	1733	—	—
1·10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	7·376
1·14	5·30	19·07	—	—	—	—	—	—	—	—	—	—	—	—	—
April 23	0·03	20·90	190	231	0·83	5·63	13·48	0·71	39·6	94·8	11·0	6·8	618	7·376	—
11·47	5·47	19·61	—	—	—	—	—	—	—	—	—	—	—	—	—
12·09	5·44	19·41	—	—	—	6·41	17·91	0·57	45·2	126·0	—	—	—	7·333	—
12·19	5·45	19·31	241	307	0·79	—	—	—	—	—	17·3	38·8	2243	—	—
12·27	5·49	19·18	—	—	—	—	—	—	—	—	—	—	—	—	—
June 10	0·04	20·93	185	236	0·78	5·63	13·33	0·69	39·9	94·5	10·5	6·6	632	7·373	7·377
12·05	5·83	19·48	—	—	—	—	—	—	—	—	—	—	—	—	—
12·27	6·11	19·22	—	—	—	6·83	18·17	0·61	48·4	128·8	—	—	—	7·311	—
12·36	6·48	19·10	186	289	0·64	—	—	—	—	—	19·0	46·3	2440	—	—
12·44	—	—	—	—	—	(7·5)	—	—	(53·0)	—	—	—	—	(7·28)	7·284
12·45	6·82	18·94	—	—	—	—	—	—	—	—	—	—	—	—	—
June 23	0·04	20·92	175	225	0·78	5·46	13·85	0·72	38·9	98·6	11·3	6·7	591	7·381	7·359
12·04	5·45	19·59	—	—	—	—	—	—	—	—	—	—	—	—	—
12·28	5·40	19·42	—	—	—	6·24	18·17	0·60	44·4	129·3	—	—	—	7·339	—
12·38	5·38	19·33	172	260	0·66	—	—	—	—	—	15·7	30·9	1970	—	—
12·47	—	—	—	—	—	—	—	—	—	—	—	—	—	—	7·325
12·53	5·42	19·16	—	—	—	6·26	17·93	0·61	44·6	127·7	—	—	—	—	—

cleaned the skin of the subject's arm with alcoholic iodine and withdrew a sample of arterial blood by puncture of the brachial artery. The whole procedure occupied about half an hour from the commencement of taking the alveolar samples. In order to prevent clotting and stop the formation of lactic acid *in vitro*, the syringe, into which 3 to 4 c.c. of arterial blood were drawn, was partly filled on March 26 with 0·5 c.c. of a solution containing 0·4 p.c. of potassium oxalate and 0·6 p.c. of sodium fluoride, and on June 10 and 23 with 0·5 c.c. of a solution containing 0·6 p.c. of potassium oxalate and 1·0 p.c. of sodium fluoride. In the latter two experiments a glass bead was placed in the syringe to aid in mixing the blood thoroughly by shaking before placing a sample in the glass electrode. Directly the blood had been withdrawn the syringe was plunged in melting ice.

The contents of the bag were then measured and the necessary apparatus transferred to the chamber. As soon as the door was closed and the CO_2 admitted the subject again seated himself in the deck chair and remained at rest for half an hour before beginning to collect the samples of alveolar air. The procedure inside the chamber was precisely the same as outside, the brachial artery of the other arm being punctured, but the total time required was diminished, since, owing to the hyperpnœa, the bag was filled in about 2 min. instead of 10. Samples of the air in the chamber were collected about 10 min. after the propeller fan was started, at the mid-points in the collection of the alveolar and bag samples, and at the end of the experiment. Delay was unavoidable between the end of the first part of the experiment and the beginning of the second part, *i.e.* when the subject seated himself on the deck chair in the chamber: in the first experiment the interval was 51 min., but it was reduced in successive experiments until it was only 30 min. in the last. The air analyses and pH determinations were done in the afternoon, the arterial blood samples being kept packed in ice.

The glass electrode was of the type described by Kerridge [1926] with a Lindemann electrometer for indicating the null point, and it was maintained at 37° in an air thermostat. Havard and Kerridge [1929] have drawn attention to the fact that if blood is placed in the warm electrode immediately after it is withdrawn from the blood vessel it shows within a minute or two a slight fall of alkalinity which is not, however, progressive if glycolysis is checked by fluoride. If pH readings are started at once they soon reach a steady initial plateau and then after a few minutes there is a rapid fall of pH to a new plateau which remains steady for a long period. The same change is shown if the blood is placed

in ice immediately after it is withdrawn from the body and after an interval transferred to the warm electrode. In our first experiment, on March 26, we took as our index of pH of the arterial blood samples the first of these two plateaux. After some further investigations we felt, however, uncertain whether we could always depend on the cooled blood attaining the temperature of the electrode in the air thermostat quickly enough, and in our subsequent experiments we therefore took as our index the second plateau, and it is for this reason that the pH values obtained with the glass electrode were higher on March 26 than in the subsequent experiments. Our practice in the later experiments was to take the syringe from the ice and put it in a beaker of water at 37° for 5 min. before transferring the blood to the electrode. We felt too that this procedure was advisable if we were to compare the direct determinations of pH with the values calculated from pH data obtained with blood *in vitro*, since in the latter case the blood had first to be brought into equilibrium with a CO_2 air mixture at body temperature and this implied maintaining it at 37° for some time. All determinations of pH were made in duplicate: these duplicates agreed closely, and only the mean value is shown in Table I. It was possible with the actual Lindemann electrometer employed to measure the potential set up by the glass electrode to 0·2 millivolts, corresponding to 0·003 pH. Although other experimental errors introduced variations larger than this duplicate determinations on the same sample of blood were frequently within 0·01 pH of each other. We have given our results to three places of decimals as the third place has some, though no very great, significance.

The results of the experiments are shown in Table I. In the observations made in pure air the data are stated without reference to the time at which the different samples were taken in order to save space, but in the observations in the chamber these times are stated, as the changing composition of the air has to be taken into account when calculating the respiratory quotients, etc.

By allowing half an hour's rest before beginning the observations we hoped not only that the respiratory exchange would have reached a steady value but that in addition the subject would have attained equilibrium with the CO_2 atmosphere in the chamber. With the rise in alveolar CO_2 pressure there would presumably be a damming back of a considerable amount of CO_2 in the tissues of the body in correspondence, and, so long as this was occurring, the respiratory quotient should be unduly low. It is possibly due to this cause that the respiratory quotient calculated from the expired air samples tends to be lower when breathing

the CO_2 atmosphere than when breathing pure air, and this difference is particularly marked on June 10 when the CO_2 concentration in the air breathed rose continuously and considerably. The difference is more obvious in the respiratory quotients calculated from the alveolar air samples. In the case of these samples a very low quotient is given even when the subject is breathing pure air, but many earlier experiments have shown that the respiratory quotients calculated from Douglas's alveolar air are always lower than when calculated from samples of his expired air.

Save in the first experiment, where the determination of the oxygen consumption made before entering the chamber shows a rather high value, the oxygen consumption when breathing the CO_2 atmosphere is higher than that shown when breathing pure air, but the difference can probably be accounted for by the extra energy expenditure entailed by the hyperpnœa. The highest figure is shown on April 23, but on this date the position of the fan in the chamber had been altered and the air current blew too directly on the subject with the consequence that he felt cold in the latter part of the experiment and was actually beginning to shiver in the last few minutes. On June 11 the hyperpnœa was getting pronounced at the time that the sample of expired air was collected owing to the rapid rise in the CO_2 concentration in the inspired air.

In the tables the volume of expired air is expressed at 37°, saturated with moisture, and at the prevailing barometric pressure so as to give a true index of the volume changes of the lungs. It will be seen that the hyperpnœa involves no great increase in the rate of the breathing; increase in the depth of the breathing plays the predominant part in determining the increased volume of air breathed. This is characteristic of a hyperpnœa caused by CO_2. On June 10 when the CO_2 reached 6·8 p.c. in the inspired air the hyperpnœa was very marked—for a CO_2 hyperpnœa, but, as is well recognized, the actual volume of air breathed in such a case is far less than when panting is caused by deficiency of oxygen in association with increase of CO_2 when the increase in the rate of the breathing may become a prominent factor [Haldane, Meakins and Priestley, 1919], or by vigorous muscular exercise (cp. the recent observations of Barcroft and Margaria [1931]).

In order to calculate the pH of the arterial blood from the existing alveolar CO_2 pressure and the curve relating CO_2 pressure and the pH of the blood *in vitro*, we have redetermined this curve on three samples of Douglas's blood taken on different dates. The blood was obtained from one of the veins in the forearm in the middle of the morning (the

subject having had his usual breakfast) after the arm had been immersed up to the elbow in warm water for some minutes so as to accelerate the circulation. Though Henderson and Haggard [1918] have shown that exposure of animals to high concentrations of CO_2 leads to an increase of the CO_2 capacity of the blood, the observations of Davies, Haldane and Kennaway [1920] indicate that in the case of man the alteration of the CO_2 capacity of the blood is inappreciable if the exposure is limited to such concentrations of CO_2 as we have used in these experiments, and we therefore contented ourselves with observations made on blood withdrawn from the subject whilst breathing normal air. From 15 to 18 c.c. of blood were withdrawn into a dry syringe containing 0·03 g. of potassium oxalate and 0·03 g. of sodium fluoride, and after being well shaken were preserved in melting ice. Saturation at 37° with different mixtures of CO_2 and air was done in the usual way, 6 c.c. of blood being used in each case. After 20 min. saturation a sample of the air in the saturator was taken for analysis and 3·5 c.c. of blood were withdrawn into a syringe. This was placed in a beaker of water at 37° and some of the blood was transferred to the glass electrode as soon as possible. The syringe was then placed in melting ice and a duplicate determination of the pH made subsequently. One c.c. of blood was also taken from the saturator and the CO_2 content determined by Haldane's [1920] ferricyanide apparatus, the apparatus being completely immersed in a water bath maintained at 20°. The results of these experiments are shown in the figure, the volume of CO_2 per 100 c.c. of blood being expressed at S.T.P.

It will be seen that the pH values obtained on the blood samples taken on May 28 and 29 fall on the same curve. The two points determined on June 19 are above this, but they appear to lie on a curve which runs parallel to the other. As it is the slope of the curve which is of importance in the subject under discussion this difference is immaterial, and the lower curve has been used for calculating the pH of the blood corresponding to the alveolar CO_2 pressures in Table I.

Direct determinations of the pH of the arterial blood taken from the brachial artery were satisfactorily obtained on March 26 and June 10 and 23. On April 23 the arterial blood samples were secured, but an error developed in the glass electrode and readings of the pH could not be obtained. On June 10 it will be noted that there was a steady rise in the CO_2 concentration in the chamber throughout the experiment. When the intended volume of CO_2 had been admitted the cock of the cylinder was by inadvertence incompletely closed; possibly there was some solid CO_2 in the orifice as the gas had been run into the chamber rather

quickly. The occupants of the chamber were unaware of this though they both commented on the fact that the hyperpnœa appeared unusually great and Douglas began to experience what he thought was a CO_2 headache just at the close of the experiment. The true state of affairs

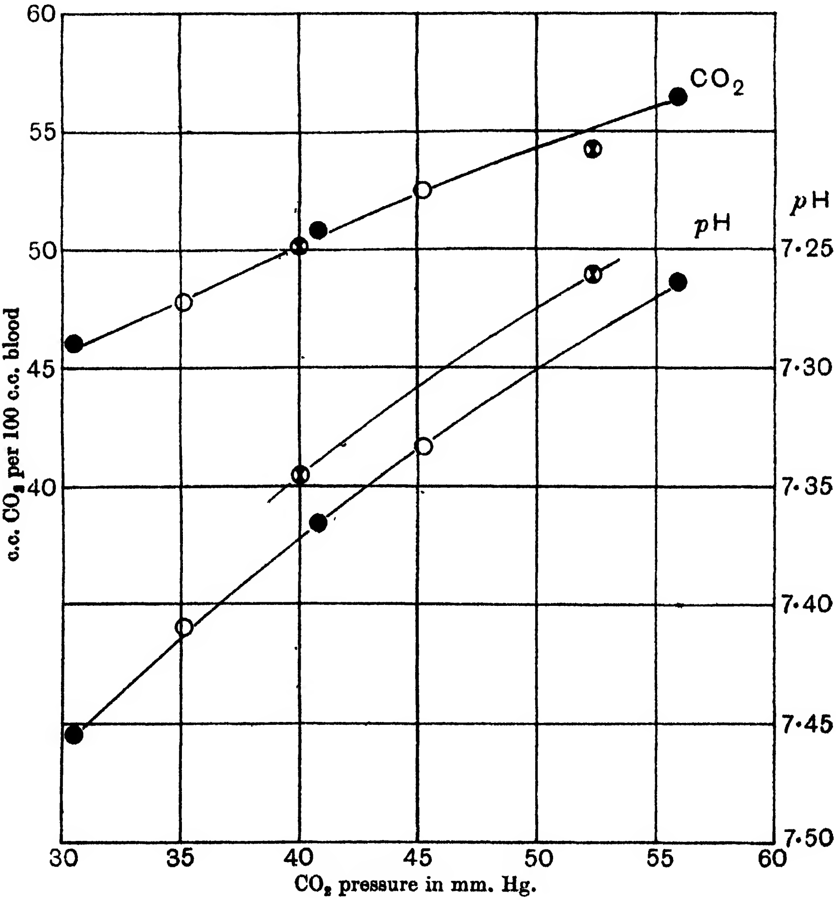

Fig. 1.

● May 28. ○ May 29. ⦶ June 19.

was only discovered after leaving the chamber. The CO_2 concentration in the inspired air was 6·11 p.c. at the mid-point of the alveolar air samples, whereas it had risen to 6·82 p.c. in the sample of air taken 1 min. after the arterial puncture, so that the pH of the blood inferred from the alveolar CO_2 concentration cannot be directly compared with the figure

given by the glass electrode. Two of the earlier experiments on Douglas which are recorded by Campbell, Douglas, Haldane and Hobson give, however, some help in this difficulty. In the first of these the resting alveolar CO_2 when breathing pure air was 5·78 p.c., while when breathing air containing 6·82 p.c. of CO_2 and 18·24 p.c. of oxygen the alveolar CO_2 was 7·68 p.c. In the second the resting alveolar CO_2 when breathing pure air was 5·54 p.c. and when breathing air containing 6·65 p.c. of CO_2 and 57·47 p.c. of oxygen the alveolar CO_2 was 7·31 p.c. (it was found that increase of the alveolar oxygen pressure did not have any material effect on the sensitivity of the subject's respiratory centre to CO_2). Judging from these results, we cannot be far wrong in assuming that in the experiment on June 10, when the alveolar CO_2 was 5·63 p.c. whilst breathing pure air, the alveolar CO_2 concentration with an inspired CO_2 percentage of about 6·8 would have been about 7·5 p.c. or 53 mm., which by reference to the curve given in the figure would correspond to a pH of about 7·28. These provisional figures are shown in the table in brackets.

The reason why the pH values obtained with the glass electrode on the arterial blood samples on March 26 are at a definitely higher level than those obtained on June 10 and 23 has already been explained. We might in any case expect some difference between the figures given by direct observation and those inferred from the alveolar CO_2 pressure and the pH CO_2 curve obtained *in vitro,* since the blood samples for the experiments *in vitro* were obtained on different dates and under different circumstances from those used for the direct determination of arterial pH, and as a matter of fact the difference on June 23 can be reduced by using the upper of the two curves (obtained on June 19) shown in the figure instead of the lower for the purpose of calculating the pH. Still this difference is not of moment in the present case: what is of importance is the decrease of pH, whether inferred or directly observed, which is associated with the increase in the breathing. This decrease, together with the increase of the breathing in litres per minute and the increase of alveolar CO_2 pressure, is shown in Table II.

TABLE II.

Date	Increase of breathing in litres at 37°, moist	Increase of alveolar CO_2 pressure in mm. Hg	Decrease of arterial pH: Inferred from pH CO_2 curve *in vitro*	Decrease of arterial pH: Directly determined by glass electrode
March 26	25·9	4·6	0·035	0·040
April 23	32·0	5·6	0·043	—
June 10	—	(13·0)	(0·09)	0·093
June 23	24·2	5·5	0·042	0·034

Taking the figures as a whole, there is as close an agreement between the inferred and observed changes of the pH in the arterial blood as one can reasonably expect, and they leave no doubt in our minds that during a CO_2 hyperpnœa the method of calculating the change in hydrogen ion concentration in the arterial blood from the alveolar CO_2 pressure and the pH CO_2 curve determined on the subject's blood *in vitro* is a valid one.

It is not clear whether or not the relationship of the change in pH in the arterial blood and the degree of hyperpnœa is within limits a strictly linear one, nor is it certain whether the excitability of the respiratory centre is the same or varies slightly from day to day. The differences which have to be determined by analysis in experimerts of this type are so small that, when allowance is made for experimental error, neither these experiments nor the earlier ones made on the same subject justify a dogmatic statement on either of these points. If we assume, at least as an approximation, that a linear relationship holds good, the experiments on March 26 and June 23 indicate that an increase of 10 litres in the total ventilation of the lungs is associated with a maintained increase of 2·03 mm. in the alveolar CO_2 pressure and a decrease of 0·015 in the pH of the arterial blood (corresponding to an increase of about $0{\cdot}015 \times 10^{-7}$ in cH), whether inferred or directly determined. The experiment on April 23 gives corresponding values of 1·75 mm. for the alveolar CO_2 pressure and 0·013 for the calculated pH, but this experiment is not quite so reliable, since the subject's metabolismand hyperpnœa were clearly increasing appreciably in the latter part of the experiment when, as mentioned above, he began to feel cold, and the alveolar air determinations were made 10 min. before the collection of the bag sample from which the ventilation of the lungs was ascertained. So far as we can judge from the data obtained on June 10, the corresponding figures must have been of the same order of magnitude—perhaps rather higher than those given by the other experiments, but it is not improbable that in this case, with the CO_2 concentration rising to 6·8 p.c. in the inspired air, we were beginning to approach the limit to which a pure CO_2 hyperpnœa can rise.

The figures derived from these experiments agree precisely with the earlier ones obtained on the same subject. Thus Campbell, Douglas and Hobson found that during a CO_2 hyperpnœa a rise of 2·0 mm. in the alveolar CO_2 pressure was sufficient to cause a rise of 10 litres per min. in the total ventilation of the lungs, and this corresponded to an increase of $0{\cdot}013 \times 10^{-7}$ in the cH of the arterial blood as judged from Hasselbalch and Lundsgaard's observations. In these experiments the

alveolar ventilation, as distinct from the total ventilation, was also calculated from the volume of each breath, the total ventilation of the lungs per minute, and the CO_2 concentration in the expired, alveolar and inspired air by making allowance for the theoretical dead space in the respiratory tract, and it was found that a rise of 2·5 mm. in the alveolar CO_2 pressure or an increase of about $0{\cdot}016 \times 10^{-7}$ in the cH of the arterial blood corresponded to a rise of 10 litres per min. in the alveolar ventilation. A similar calculation made on the data obtained on March 26 and June 23 in the present series gives an almost identical result, an increase of 10 litres per min. in the alveolar ventilation being accompanied by a rise in the alveolar CO_2 pressure of about 2·4 mm. and a fall in the pH of the arterial blood of about 0·018 (*i.e.* a rise of about $0{\cdot}018 \times 10^{-7}$ in the cH).

It is a point of some interest that the sensitivity of Douglas's respiratory centre to changes in the arterial CO_2 pressure or hydrogen ion concentration during a CO_2 hyperpnœa shows no appreciable alteration after the lapse of seventeen years.

It is instructive to compare the very small change of hydrogen ion concentration in the arterial blood which we have shown to be correlated with a given increase in the ventilation of the lungs during a CO_2 hyperpnœa with similar data obtained under other conditions by different observers.

During moderate muscular work of 4600 ft. lb. (640 kg. m.) per min. on the bicycle ergometer, T. R. Parsons, W. Parsons and Barcroft [1920] found that an increase in lung ventilation of about 21 litres per min. (measured at room temperature) was accompanied by a decrease of pH in the arterial blood from 7·43 to 7·35, the figures being inferred from a comparison of the existing alveolar CO_2 pressure with the pH CO_2 pressure curve determined *in vitro* on a sample of the subject's completely reduced blood with the aid of the hydrogen electrode. In this instance therefore a rise of 10 litres in the total ventilation of the lungs corresponds with a decrease of about 0·04 in the pH of the arterial blood, as opposed to a decrease of about 0·015 found in our experiments during CO_2 hyperpnœa. Arborelius and Liljestrand [1923] also investigated the change of pH in the arterial blood during moderately hard muscular work on the bicycle ergometer, the maximum work being 945 kg. m. per min. for Arborelius (= an oxygen consumption of about 2300 c.c. per min.) and 756 kg. m. per min. for Liljestrand (= an oxygen consumption of about 1900 c.c. per min.). The pH of the arterial blood was inferred from the existing alveolar CO_2 pressure (calculated, not directly determined)

and the *p*H CO_2 pressure curve calculated by the Henderson-Hasselbalch equation from data obtained on the subject's blood *in vitro*. They found that on the average an increase of 10 litres in the total ventilation corresponded with a fall of 0·019–0·018 in the calculated *p*H. Bock, Henderson and others [L. J. Henderson, 1928] have examined the changes occurring in the blood as the result of exercise. Their subject worked on the ergometer for long enough to attain a steady state, the oxygen consumption being 1750 c.c. per min., and blood was withdrawn whilst the exercise was maintained. The calculated decrease in *p*H in the arterial serum was 0·074 and in the arterial cells 0·062. Though data of the actual volume of air breathed are not given, it would appear probable that the change of *p*H relative to the increase in the ventilation of the lungs was of the same order as that found by Arborelius and Liljestrand.

It will be seen that in each of these instances the decrease of *p*H associated with a given increase in the breathing is definitely greater than that found in our experiments on CO_2 hyperpnœa. Small differences may perhaps be attributable to definite differences in the sensitiveness of the respiratory centre of different individuals, of which the observations of Campbell, Douglas, Haldane and Hobson give some indication, but the discrepancy becomes much greater if the effects of severe muscular exercise are examined. Thus Barcroft and his colleagues [1914], basing their calculations on the shift of the oxyhæmoglobin dissociation curve, found that an ascent of 1000 ft. in half an hour resulted in a change in the *p*H of the blood from 7·29 to 7·09, a degree of change which, judging from our own experiments, would be quite out of proportion to the probable hyperpnœa. Very large changes in the *p*H of the blood as the result of severe muscular exercise have also been recorded by Barr, Himwich and Green [1923], but perhaps the most interesting of these observations is that discussed by Barr [1923].

In this case the subject did severe work on the ergometer for $3\frac{1}{2}$ min. during which time a total of 4000 kg. m. was reached. The *p*H of the arterial blood was calculated from the Henderson-Hasselbalch ratio. The resting ventilation of the lungs before the exercise was 8 litres per min. During the last minute of the exercise the ventilation had risen to over 80 litres per min., the arterial *p*H had fallen from 7·35 to 7·27. In a second experiment of an identical character the arterial *p*H was 7·30 before the muscular exercise, 1 min. after resuming rest it was 7·16 (ventilation 56 litres per min.) and 3 min. after resuming rest it was even lower—7·15 (ventilation 34 litres per min.). In a third experiment of

the same type the arterial pH 3 min. after resuming rest was 7·19, and 15 min. after resuming rest 7·23 (ventilation 11 litres per min.). Taking this group of experiments as a whole, the results appear to be anomalous, for the hyperpnœa caused by the muscular work is subsiding while the hydrogen ion concentration of the arterial blood is increasing, and even when the ventilation of the lungs has diminished 15 min. after the stop of the exercise to a value only 3 litres in excess of the normal resting ventilation the arterial pH is still far below (0·07–0·12) the initial resting figure. From our results given in this paper we might have expected a persistent intense hyperpnœa. Such a result as this is reminiscent of some earlier conclusions reached by Hasselbalch [1916]. Taking as his data some observations on the CO_2 combining power of the blood collected 5–8 min. after a short period of violent muscular exertion which were published by Christiansen, Douglas and Haldane [1914], he calculated from the ratio of free to combined CO_2 that the pH of the blood was at that time 7·21, whereas it was 7·33 when the subject was at rest before the exertion. From our results a fall of 0·12 in the arterial pH might be expected to be accompanied by an increase in the ventilation of the lungs of 80 litres per min. above the normal resting value. In point of fact the subject himself thought that the hyperpnœa had died down by the time that the blood sample was taken, and calculation from his alveolar CO_2 concentration shows that the ventilation of the lungs was actually only about 25 p.c. above the resting value.

Results of this type may well, and indeed have, aroused grave misgivings as to the precise relationship between the hydrogen ion concentration of the blood and the activity of the respiratory centre. The Henderson-Hasselbalch equation on which many of the calculations of blood pH are based may not hold good without, say, some modification of the constant if results obtained at rest are to be compared with the alteration of conditions imposed by severe work. That is a possibility that must, it is true, be faced, but that it cannot at most be more than a minor factor seems to be clearly shown by the very striking results recorded by J. B. S. Haldane, Linder, Hilton and Fraser [1928]. They investigated the condition of the blood in a resting subject during a severe acidosis caused by the ingestion of large quantities of ammonium chloride. At the height of the acidosis the pH of the arterial blood obtained from the femoral artery was found to be 7·29 when it was determined by the colorimetric method of Hastings and Sendroy, whereas it was 7·41 before the ammonium chloride was taken. The alveolar CO_2 pressure under normal conditions was 34·8 mm., and at the

height of the acidosis 18·3 mm., which would imply that the acidosis was associated with an increase of about 90 p.c. in the resting ventilation of the lungs, *i.e.* though the resting breathing was barely doubled the *p*H of the arterial blood had fallen by no less than 0·12.

These experiments on severe muscular work and on ammonium chloride acidosis are alike in showing a vastly greater fall of arterial *p*H for a given increment in the pulmonary ventilation than we should expect from our observations on CO_2 hyperpnœa. Is there any way of reconciling these differences? We feel that there is one line of argument that is well worth consideration, an argument that has been outlined earlier in this paper.

If the intracellular hydrogen ion concentration is the factor which counts in the determination of the activity of the respiratory centre, we are bound to consider the extent to which changes in arterial *p*H can affect the intracellular reaction, and that must depend on the ease with which changes in the *p*H of the blood can be propagated through the cell membrane. In our experiments the reduction of arterial *p*H is due to an increase in the free CO_2, and, according to Jacobs, the cell membrane is freely permeable to CO_2, though this is not the case with other molecules or ions. We might therefore expect to cause an immediate change of intracellular reaction in correspondence—possibly this change would be identical in magnitude with that found in the arterial blood, that would depend on intracellular conditions. But when we take the case of a reduction of arterial *p*H due to severe muscular work or ammonium chloride we have a quite different state of affairs. Take, for instance, Barr's experiments of which we have given details. Severe muscular work of the type he employed floods the body with lactic acid. When the work stops the *p*H of the blood is low and the bicarbonate, owing to neutralization by lactic, acid is greatly reduced. The figures show that the arterial *p*H continues to fall for a minute or two though the hyperpnœa at once begins to subside. That the *p*H should continue to fall after a brief period of muscular exertion is not surprising; presumably this means that excess of lactic acid formed during the muscular activity is still escaping from the muscles into the blood stream and getting distributed throughout the body. We know, however, that when we resume rest after such a muscular exertion the alveolar CO_2 pressure falls to a figure much below normal and that the normal value is only gradually regained as lactic acid is eliminated. A minute or two after the stop what is the condition? We have a very low *p*H accompanied by a great reduction in bicarbonate and a diminution in the free CO_2 in

the arterial blood. If the cells of the respiratory centre are freely permeable to CO_2 but relatively impermeable to other factors which contribute to the hydrogen ion concentration, the reaction of the interior of the cells of the respiratory centre is not necessarily influenced by a change of arterial *p*H to the degree that we might otherwise expect. It may be that we ought also to reckon with some alteration in the ionic equilibrium between the interior of the cells and the blood which is governed by the Donnan ratio. Taking these possibilities into account, it seems to us not improbable that at the moment in question the increase in hydrogen ion concentration above the normal resting level may have been considerably less within the cells than in the arterial blood. If that were so, the diminution of the hyperpnœa is intelligible from the theory of the regulation of the breathing by hydrogen ion concentration. It is quite possible that a similar discrepancy, though less in degree, between the arterial and intracellular *p*H may have existed in the experiments on moderate muscular work which have been quoted above since in most, if not all of these, there is evidence of some accumulation of lactic acid as a result of the exercise. It may be added that in ammonium chloride acidosis there may be a low arterial *p*H accompanied by a low alveolar CO_2 pressure, implying a diminution in the free CO_2 in the arterial blood, and a great reduction in the CO_2 combining power of the blood, but these facts tell us nothing directly about the reaction within the cells of the respiratory centre.

The statement that the activity of the respiratory centre is dependent on the *p*H of the arterial blood which reaches it may be perfectly true as a generalization, but when once the question of the permeability of the cell membrane is called in question we have no right to assume that temporary and violent fluctuations of arterial *p*H must of necessity cause a simultaneous and identical variation of *p*H in the cells of the respiratory centre.

Summary.

1. The change in *p*H in the arterial blood accompanying hyperpnœa caused by breathing air containing CO_2 has been directly determined by means of the glass electrode in one subject. In addition, the change of alveolar CO_2 pressure has been determined and the change of *p*H calculated from this value and the curve relating CO_2 pressure and *p*H in the same subject's blood when this is exposed to different concentrations of air and CO_2 *in vitro*.

2. The direct determinations of pH agree precisely with the calculated values.

3. Within the limits of CO_2 concentration covered in these experiments an increase of 10 litres per min. in the total ventilation of the lungs was associated with a rise of about 2·0 mm. in the alveolar CO_2 pressure (*i.e.* in the arterial CO_2 pressure) and a decrease of about 0·015 in the pH of the arterial blood. An increase of 10 litres per min. in the calculated alveolar ventilation was associated with a rise of about 2·4 mm. in the alveolar CO_2 pressure and a decrease of about 0·018 in the pH of the arterial blood.

4. The sensitivity of the subject's respiratory centre to changes of arterial CO_2 pressure and pH accompanying a CO_2 hyperpnœa is the same as it was seventeen years ago.

5. The discrepancy between these results and the figures recorded by other observers for the change of arterial pH associated with a given increment of the breathing under conditions of muscular work and in ammonium chloride acidosis is discussed. It is suggested that this discrepancy may perhaps be accounted for by the fact that large fluctuations of arterial pH need not necessarily cause simultaneous and identical changes of intracellular pH in the respiratory centre.

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THE EFFECTS OF INTESTINAL RHYTHM ON GENERAL BLOOD-PRESSURE.

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RECENTLY Barcroft and Nisimaru [1932] have shown that alternate rhythmic contractions and relaxations of the spleen may be at least partially responsible for the rhythmic fluctuations often appearing on blood-pressure tracings resulting from the stimulation of the vagus, sciatic and splanchnic nerves, the injection of adrenaline, curare and histamine, and the temporary stoppage of respiration and of the splenic circulation. It was also noted that the rhythmic contractions of the spleen could be initiated by a sudden rise of blood-pressure after denervation, removal of the suprarenals, and by perfusion of the isolated organ.

Now since this organ may be responsible for such striking rhythmic effects on blood-pressure, a decrease in spleen volume causing a rise in blood-pressure, it was of interest to find whether the intestine also possessed a similar rhythmic property which is capable of altering blood-pressure simultaneously. The only previous literature we were able to find concerning this problem was that of Bunch [1899], who presents one curve in conjunction with many others concerning the vaso-motor supply of the intestines. Although this curve is quite similar to those given in this paper, it lacks a time tracing and any detailed explanation; as a result we feel justified in presenting curves concerning some of the different ways in which this intestinal rhythm may be set up and also giving evidence that its origin is in the intestine itself and not primarily under nervous or hormone control.

METHOD.

All the experiments were carried out on large cats (2·5 kg. or over). They were anæsthetized with 25 p.c. urethane (4 c.c. per kg.) 3 hours before the experiment. The oncometer used was of the air conduction

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type with a membrane manometer for recording volume changes. Before placing the intestine in the oncometer, the duodenum was ligated and cut about 2 in. from the pylorus. Similarly the colon was ligated and cut about 4 in. from the cæcum, care being taken to tie off any small blood vessels in the region of the section. This left practically the whole intestine free to be placed in the oncometer with the exception of the region where the superior mesenteric artery and vein make their connection between the intestine and the rest of the animal.

The oncometer was adjusted above the animal's abdomen so as to avoid any undue pull or pressure on the vessels. The transparent cover of the oncometer was sealed with vaseline and metal clips, and the temperature kept as nearly constant as possible by electric heating devices. It must be emphasized that the greatest possible care in keeping the animal in good condition is necessary to obtain the most satisfactory results.

Experimental.

The experiments carried out on this preparation were all very similar in type to those made by Barcroft and Nisimaru while working on the spleen. It was necessary to do this in order to carry out our original intention of noting whether any similarity existed between the types of intestinal rhythm and rhythm of the spleen. Therefore in most of the following experiments duplicates were performed—one with the splenic circulation still intact, and another in which the splenic artery was clamped, which gave us the necessary information as to whether the intestinal rhythm was independent of that of the spleen, and also whether they were in any way similar.

The different experiments performed in this way were as follows: stimulation of the central end of the cut vagus, sciatic, and left splanchnic; temporary stoppage of respiration and clamping of the mesenteric artery; injection of small doses of adrenaline and curare. In some cases experiments were also done after the intestines were completely denervated, and the suprarenals tied off.

In all the curves which are to be given it will be noticed that these different methods seem to set up a characteristic intestinal rhythm, with a corresponding effect on blood-pressure, the duration of the wave varying from 21 to 47 sec.; the variation was, no doubt, due to the condition of the intestines, *i.e.* the better the condition of the intestines, the quicker the rhythm. To show the range in the rhythms in the various experiments we have prepared the accompanying table; a glance will

show that the frequencies of the intestinal and blood-pressure rhythms are identical.

TABLE I.

Cat No.	Duration of wave of intestinal rhythm (sec.)	Duration of wave of blood-pressure rhythm (mercury manometer) (sec.)	Technique used in performing experiments
11	35	35	Clamping mesenteric artery (spleen in system)
11	35	35	Clamping mesenteric artery (spleen out of system)
14	21	21	Stimulation of central end of vagus (spleen in system)
14	21	21	Stimulation of central end of vagus (spleen out of system)
15	23	*	Stimulation of central end of vagus (spleen out of system, intestines denervated, weak stimulating current)
15	23	*	Stimulation of central end of vagus (spleen out of system, intestines denervated, medium stimulating current)
15	27	*	Stimulation of central end of vagus (spleen out of system, intestines denervated, strong stimulating current)
14	27 average	No noticeable effect	Stimulation of central end of sciatic after curare injection (spleen out of system)
17	35	35	Stimulation of l. splanchnic (spleen in system)
17	35	35	Stimulation of l. splanchnic (spleen out of system)
17	35	35	Stimulation of l. splanchnic (spleen out of system, suprarenals tied off)
11	25	25	Clamped trachea after vagi cut and curare injected (spleen in system)
11	25	25	Stopped artificial respiration after vagi cut and curare injected (spleen out of system)
11	25	25	Stopped artificial respiration after vagi cut and curare injected (spleen out of system, intestines denervated)
14	28	28	Injected adrenaline (spleen out of system)
14	33–47	33–47	Injected curare (spleen out of system)

* The failure of blood-pressure effects was due to a defective membrane manometer used on this particular day.

Now since it will be impossible to present all the curves obtained in this type of work, only a limited number of the most characteristic experiments will be presented.

Exp. 1. In this experiment we cut and stimulated the central end of the left vagus, the splenic artery having been previously clamped. The general blood-pressure was 118 mm. of Hg, and during the stimulation with Faradic current for 15 sec. the blood-pressure fell 26 mm. Hg with a corresponding increase in intestinal volume of approximately 0·8 c.c.

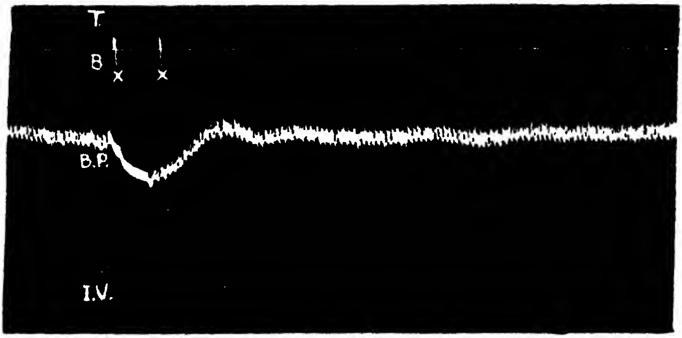

Fig. 1. *T.* time, 5 sec. *B.* base line at 160 mm. Hg. *B.P.* general blood-pressure, carotid artery. *I.V.* intestinal volume. *x–x*, stimulation of the central end of the left vagus.

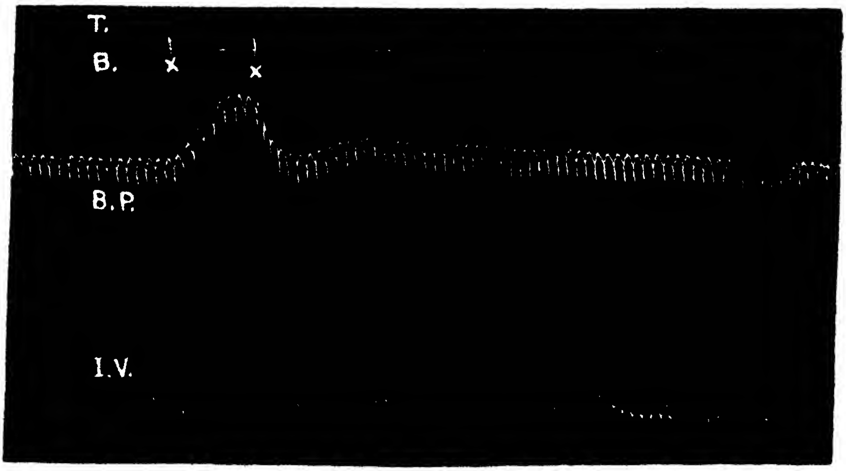

Fig. 2. *T.* time, 5 sec. *B.* base line at 160 mm. Hg. *B.P.* general blood-pressure, carotid artery. *I.V.* intestinal volume. *x–x*, stimulation of the central end of the sciatic nerve.

After cessation of the stimulus the blood-pressure and intestinal volume returned practically to normal within 15 sec., after which the characteristic rhythm of the intestine was set up with a corresponding undulatory effect on blood-pressure, a decrease in intestinal volume causing an increase in general blood-pressure (Fig. 1). An exact duplicate of this tracing was produced, as far as blood-pressure and intestinal rhythm are concerned, before the splenic circulation was removed.

Exp. 2. Exp. 2 is concerned with the effects of stimulating the central end of the sciatic nerve. In this case the animal was previously injected with curare, and artificial respiration was given. The spleen also had been previously removed from the circulatory system. The general blood-pressure was 103 mm. Hg at the start, and upon stimulation for 25 sec. rose 35 mm. with a decrease in intestinal volume of about 0·5 c.c.; a return to normal occurred within 10 sec., and was followed by an intestinal rhythm, but with only a very slight trace of an effect on general blood-pressure (Fig. 2).

Exp. 3. Here we show the effects of stimulation of the left splanchnic nerve after the splenic artery had been clamped and the suprarenal had been tied off on the same side as the stimulation. The general blood-pressure was 84 mm. Hg, with a rise in pressure of

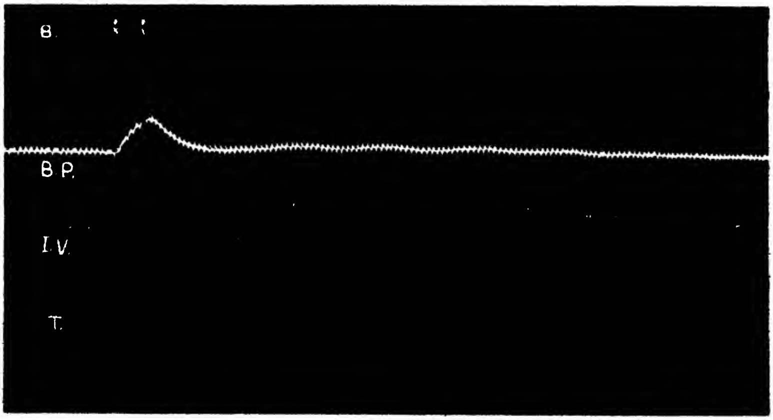

Fig. 3. *T.* time, 5 sec. *B.* base line at 170 mm. Hg. *B.P.* general blood-pressure, carotid artery. *I.V.* intestinal volume. *x–x*, stimulation of the left splanchnic nerve.

20 mm. resulting from a Faradic stimulation for 10 sec. The intestinal volume decreased about 1·6 c.c. After stimulation the recovery in blood-pressure took 22 sec.; the intestinal volume returned to normal in 55 sec., and, following a slight increase in volume above normal, rhythmical variations of 35 sec. period and simultaneous fluctuations in blood-pressure were evident (Fig. 3).

Exp. 4. This experiment shows the effects of stoppage of respiration. Before performing this experiment the cat was paralysed with 2 c.c. of 1 p.c. solution of curare, and artificial respiration was given. Also both vagi were cut. General blood-pressure was 67 mm. Hg before stopping artificial respiration. During this stoppage of about 2 min., the blood-pressure gradually rose for about 1 min. and then fell, gradually at first, but later more rapidly. During that time the intestine showed a very slight rhythm. Upon restarting respiration, a pronounced rise in blood-pressure was noted with a similar rise in intestinal volume; but directly afterwards the intestine showed a strong contraction for a short period, which was followed by the usual rhythm of about 25 sec. period, as shown in Fig. 4. Also an undulatory effect in blood-pressure may be noticed. This effect was produced with the spleen in the system, but similar alterations in blood-pressure and intestinal volume

were produced with the splenic artery clamped, and in another case, after the intestines were denervated. It may be mentioned here that we also obtained an intestinal rhythm following a temporary clamping of the mesenteric artery for about 30 sec.

Exp. 5. In this experiment 0·025 mg. of adrenaline was injected into the cat, the spleen having been clamped off. The result was a rise of 32 mm. Hg in blood-pressure over that of normal (112 mm.) with a corresponding decrease in intestinal volume. But it will be noticed

Fig. 4. *T.* time, 5 sec. *B.* base line at 0 mm. Hg. *B.P.* general blood-pressure, carotid artery. *I.V.* intestinal volume. *x–x*, stoppage for respiration.

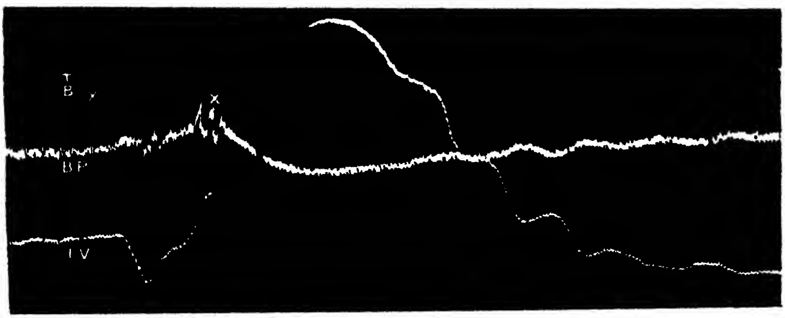

Fig. 5. *T.* time, 5 sec. *B.* base line at 160 mm. Hg. *B.P.* general blood-pressure, carotid artery. *I.V.* intestinal volume. *x–x*, time for injection of adrenaline.

in Fig. 5 that subsequently the intestinal volume increased markedly above its normal value for a period of 1½ min., and then gradually recovered to its initial volume, with the usual series of rhythmic waves. During this time the blood-pressure fell, but only slightly compared with the volume increase of the intestine. Upon recovery the usual fluctuations in blood-pressure were discernible on the tracing.

Exp. 6. In the last experiment to be reported, the effect of curare on intestinal rhythm was recorded. The general blood-pressure of the cat was found to be 96 mm. Hg, after which 1 c.c. of 1 p.c. curare was injected into the femoral vein. As will be noticed, the blood-pressure gradually decreased 34 mm. with a corresponding increase in intestinal volume. The rhythm was at first of 47 sec. period, but was followed by a shorter rhythm of 30 sec. period as shown in Fig. 6.

Now since Barcroft and Nisimaru in previous experiments on the spleen reported a rhythm with period varying from 20 to 80 sec. and our experiments on the intestine, performed in the same manner, yielded only rhythms with a period of from 21 to 47 sec., the question would naturally arise whether we are justified in our previous statement that they are similar. To prove this similarity we performed experiments recording the intestinal volume while the splenic artery was clamped temporarily and then released. In this case we found an undulatory change in blood-pressure with no effects on the intestinal volume. We assumed that this

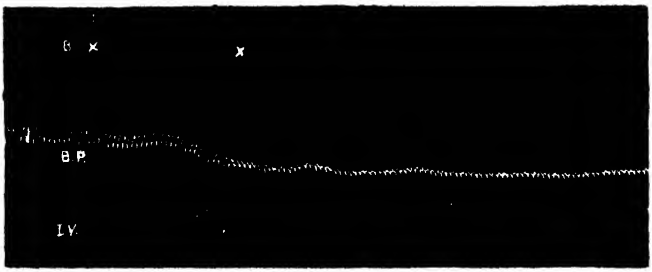

Fig. 6. *T.* time, 5 sec. *B.* base line at 160 mm. Hg. *B P.* general blood pressure, carotid artery. *I.V.* intestinal volume *x–x*, time for injection of curare.

rhythm was produced by the spleen. Then, by clamping the splenic artery and producing a rhythm of the intestine, as shown in our tracings, we were able to find a rhythm of exactly the same period as that produced by the spleen, as shown in Table II. This would indicate that the rhythm

TABLE II.

Cat No.	Period of rhythm of spleen	Technique used	Period of rhythm of intestine	Technique used
17	35	Clamping splenic artery	35	Stimulation of splanchnic
11	30	Clamping splenic artery	30	Clamping mesenteric artery

of a cat's intestine is harder to record than that of the spleen, because the latter has a stronger contracting power. This may account for the inability to record an intestinal rhythm with a period which is longer than 47 sec.

Discussion.

We believe that these experiments give evidence that the intestines have a rhythm which is of the same character as that of the spleen for the following reasons: the same procedure gives a rhythm either in the intestine or spleen and these rhythms have the same duration when under the same conditions; also the rhythm of both organs has the power of causing a similar undulatory change in general blood-pressure—that is, an increase in the volume of the organs produces a simultaneous fall in blood-pressure. The only noticeable difference between the two rhythms is that the rhythm of the intestine is smaller in amplitude than that of the spleen (roughly, about one-third in size), and therefore requires a greater magnification by the writing lever to show its undulations. As to the reason for this difference, probably the intestinal rhythm depends only on rhythmical variations in vaso-constriction in the intestine, whereas in the case of the spleen, the rhythm is associated also with rhythmical variations in the amount of stored blood.

That the rhythm of the organ is responsible for the alternate fluctuation in blood-pressure was clearly seen while performing the experiments. It may also be pointed out that if blood-pressure variations were responsible for the rhythmic changes in the intestine, one would expect a passive effect on the intestinal volume, a rise in blood-pressure causing a corresponding rise in intestinal volume and *vice versa*; but in our experiments this clearly was not the case.

As to the exact cause and nature of this rhythmic change appearing in the intestine as well as in the spleen, when the blood-pressure is altered, little definite information can as yet be given; but we believe that it has its origin in the organ itself, because the same results can be obtained after the organ is denervated, the adrenals removed and, in the case of the spleen, when it is artificially perfused. The question still remains, however, whether this rhythm is related to rhythmic variations of the smooth muscle of the organ or is due to the blood vessels of that organ.

It is also evident that this rhythm may be initiated either by impulses from the central nervous system, chemical stimulation, or mechanical stimulation. The implication, in the first case, is that, though the stimulus comes from the central nervous system, the rhythm itself is peripheral and is a property of the muscle, or local neuro-muscular complex.

Conclusions.

1. The intestine possesses the same kind of rhythm as the spleen, the duration of the wave being about 20–50 sec., as recorded by plethysmographic methods.

2. These rhythmic contractions and relaxations of the intestine have a simultaneous effect on general blood-pressure. This is less marked than in the case of the splenic rhythm.

3. These rhythmic contractions of the cat's intestines can still be recorded after removal of the spleen and adrenals, also after denervation of the intestines.

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Infra-red rays and ventilation. By LEONARD HILL.

Rays from a dull red or dark source of heat falling on the skin of the face or body, excite reflexly a congestion of the nasal mucous membrane, which narrows the air-way. Cooling the irradiated part or face by means of a fan, or by approximation of a cold surface, prevents the reflex effect. Cooling of any other part is ineffectual. Rays from a bright red source, giving a sufficiency of red and short infra-red rays, antagonize the action of the long infra-red rays. After previous warming of an area of skin by such a source, the reflex effect of the long infra-red rays is much delayed, and it takes time for this delaying influence to pass off. This antagonistic action can be provoked from the same part of the skin as, or from any part other than, that from which the reflex is provoked. An area of skin as small as 8 sq. cm. sufficed for exciting the reflex when this is exposed to a dull red electric fire. The reflex could be provoked in a sensitive subject even at a distance of 47½ ft. when the subject was facing this fire in a well ventilated room.

Breathing the warm air coming off such a fire through a hole in a screen into which the nose is thrust, does not excite the reflex. While long infra-red rays are absorbed by a film of water and do not penetrate the epidermis, short infra-red rays penetrate this as red rays do, and may excite, therefore, a different set of nerve-endings. Water vapour screens off long infra-red rays, hence the custom, hitherto unexplained, of putting a bowl of water in front of a dark or dull red source of heat.

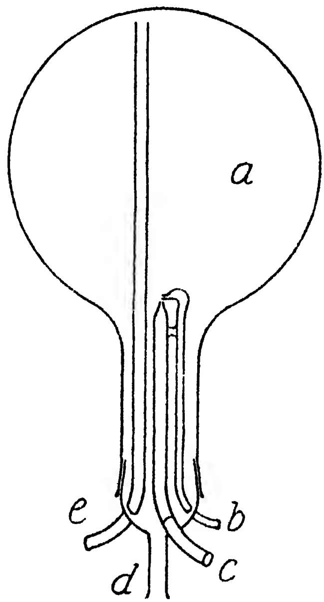
The stuffy effect felt in closed rooms appears to be due largely to the reflex effect of the long infra-red rays on the nose, and are set aside by adequate ventilation of the face with cool air.

To demonstrate the above effects one nostril is closed by a plug of wool. Breathing through the other nostril then becomes difficult on exposure of the skin to an electric fire, that is in those subject to catarrh and a stuffy nose. In others with widely open noses a screw nose clip can be used to partially obstruct the air-way beforehand. Such people are relatively insensitive to the discomfort of dark heat rays.

An oxygenator for perfusion experiments. By U. S. von Euler[1] and C. Heymans. (*Institut J. F. Heymans, Department of Pharmacology, University of Ghent.*)

A number of artificial oxygenators for perfusion experiments have been used and described, among which those of Bayliss, Fee and Ogden [1928] and Bornstein [1926] are founded on two often used systems.

At the suggestion of C. Heymans one of us (U. S. v. E.) has used in a series of perfusion experiments an apparatus, based on the principle of spreading out the perfusion fluid in fine drops by means of the gas required, originally constructed for other purposes [J. F. Heymans, 1919; J. F. and C. Heymans, 1926]. Fig. 1 illustrates the oxygenator as employed in the experiments referred to in this note.



In a glass balloon (*a*) of 5–10 litres volume a glass stopper is tightly fitted, through which the tubes for inflowing fluid (*b*) and gas (*c*) and the out-flow tube for the fluid (*d*) are running. The tubes for gas and fluid (*b* and *c*) are arranged in the usual way so as to cause spreading out of the fluid into fine drops. The glass stopper may also be fitted with an extra outlet tube (*e*) for the gas. Instead of the glass stopper an ordinary rubber bung carrying the tubes may be used, as in the original description.

[1] Fellow of the Rockefeller Foundation.

The openings of the mouthpieces of the tubes for gas and fluid were about 1–1·5 mm. each in diameter. Preferably the mouthpieces are exchangeable.

In the perfusion experiments, for the purpose of which this oxygenator was employed, defibrinated blood, after it had been allowed to stand in the ice-box for some 24 hours in order to inactivate the vasotonins [Bornstein, 1926], was used at a flow of up to 250 c.c. per minute and with an oxygen consumption of up to 20 c.c. per minute. These figures are not to be regarded as maximum figures as the capacity may be increased by suitable adjustments of mouthpieces and by changing the volume of the glass flask. The gas employed was either pure oxygen from a gas cylinder or ordinary air from a small air pump[1] which would as a rule give sufficient oxygen saturation of the blood. Preferably a mixture of air and CO_2 in order to avoid acapnœa should be used. Drying up of the finely divided blood in the oxygenator was avoided by letting the gas pass in fine bubbles through a pierced rubber bag in a bottle of heated water. The effectiveness of this arrangement was proved by testing the oxygen capacity of the blood which remained practically unaltered for 2 hours.

No new formation of vaso-constrictor substances following the passing of the blood through the oxygenator was observed.

With proper handling, the spreading out of the defibrinated blood in fine drops did not cause frothing as might have been feared. Hæmolysis occurred to a slight degree only in some cases where the "pulverization" was unusually hard or if blood considerably older than 24 hours was used. If the "pulverization" is so intensive as to give complete saturation with air at the temperature of the blood in the oxygenator, which may be considerably lower than that of the rest of the circulating blood (about 38°), gas may be liberated as the blood passes through the thermostat. Thus fine gas bubbles, just visible to the naked eye, may be noticed in the blood flowing to the preparation under these circumstances. This disadvantage can be easily avoided by diminishing the gas stream or by keeping the oxygenator at the temperature of the blood in the thermostat.

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[1] The air pump used was an "Atmos-Pumpe, Sauerstoff-Centrale, Berlin N.W. 6," with a capacity of about 4·5 litres per minute.

Degeneration of the boutons terminaux in the spinal cord.
By E. C. Hoff.

Using a perfected method of fixation of the central nervous system of the cat, namely, the injection of 10 p.c. chloral hydrate into the living anæsthetized animal, it has been possible to stain all the *boutons terminaux* (Cajal) with consistent results. Preparations of these nervous terminals in the cord, cerebellum, and cerebral cortex were demonstrated at the Oxford meeting of the Physiological Society, 1931.

With this technique, experiments were undertaken to determine the effect upon the boutons of cutting afferent roots to the lumbar and cervical enlargements of the cord. Adult cats were deafferented on the right side at the fifth, sixth, seventh and eighth lumbar roots; and others at the right fourth, fifth and sixth roots of the cervical enlargements. The animals were killed at daily intervals from 24 hours to two weeks after the operation.

In the 24-hour cats, the boutons of the cells of a restricted, clearly defined region in the dorsal part of the grey matter show pronounced modifications. They are enlarged, somewhat elongated, and noticeably swollen. At 48 hours, the boutons are more swollen, granular, enlarged, and elongated; and instead of showing the loop-like appearance of the normal terminal, they are completely solid. In the case of the three-day cats, the degeneration is more pronounced, and some terminals are almost entirely obliterated. At four days, few boutons are to be found on these cells, and there is no trace of an abnormal bouton in the six-day animal. No degenerating terminals have been found at any stage upon the ventral horn cells in either the lumbar or cervical enlargements. The boutons on these cells remain perfectly normal. The entire left or control side is always found to be normal.

With the view of determining whether the boutons on the cells in other parts of the cord would degenerate, transverse semisections were made in the cat's cord. In the animals killed at intervals of from 24 hours to four days, the same modifications have been observed in the boutons of the cells adjacent to the lesion: the terminals enlarge, elongate, become swollen and granular, and finally disappear.

These experiments demonstrate that separation of the nerve fibre from its cell body is followed by a degeneration of its termination in the grey matter which can be observed, in the cat, as early as 24 hours after the section, and is complete in four days. The experiments also add a final proof of the nervous nature of the *boutons terminaux*.

They suggest, further, that afferent fibres do not terminate directly around ventral horn cells, but that the simplest ipsilateral reflex involves at least one internuncial neurone. Finally, afferent fibres end on cells of the same side, and never cross to terminate on cells of the opposite side.

Uterine fistula used to determine the mechanism of ascent of the spermatozoon in the female genital tract. By H. FLOREY and A. WALTON.

Uterine fistulæ have been established in rabbits, rats and guinea-pigs. Females which were not pregnant and had been separated some time from males were used. After shaving and disinfection of the skin with a weak tincture of iodine, the abdomen is opened by a median incision. The uterine horn on one side of the body is secured by means of a retaining ligature passed through the mesentery. A short distance from the median incision and on the same side as the retained horn, a small incision is made. By means of the retaining ligature a short segment of the uterus is pulled into the incision, care being taken not to distort the uterus or strangulate any of the bowel. The large median incision is now closed. The segment of uterus is first secured with cat-gut to the muscle coat and the retaining ligature removed. It is then opened by means of a longitudinal incision and the free edges stitched to the skin with horse-hair. The whole operation is carried out with strict asepsis. No dressing is put on the wound. In a few days the stitches can be removed and the lumen cleared of any clots or necrotic tissue. In about a week the skin grows round the opening, leaving a small aperture large enough to insert a probe or small pipette. The aperture tends to close with time but can be kept open by passing a probe daily. The animal can now be used for experiment.

Since the rabbit does not ovulate spontaneously it can be used as soon as the wound has healed. It is mated to a vigorous and fecund male. At stated intervals after copulation a fine pipette, moistened with Ringer's solution is introduced into the lumen of the uterus through the fistula and withdrawn with slight suction. In this way a small sample of the uterine contents is obtained. Spermatozoa in small numbers at first, but increasing gradually, appear 20 min. to 2 hr. from copulation. The interval increases with the distance of the fistula from the cervix. Some of the animals were killed shortly after copulation. The distribution of spermatozoa in the fistulated and intact horns was approximately the

same, showing that the operation had not interfered with normal functioning of the horn. The results confirm our previous conclusion that in the rabbit spermatozoa find their way into the uterus by gradual penetration and not rapidly by uterine "suction" or peristalsis.

Results with rat or guinea-pig are entirely different. The animals were mated when "on heat" as determined by the vaginal smear. Immediately after copulation undiluted semen containing masses of spermatozoa may appear at the open fistula. In these animals the spermatozoa are undoubtedly transported mechanically. We believe that the principal factor involved is a spasmodic contraction of the vagina. The presence of the copulation plug prevents the escape of the semen backwards and the whole force of the contraction will be directed forwards into the uterus. (A copulation plug does not form in the rabbit.)

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Phosphagen and cardiac function (2). By A. J. CLARK, M. G. EGGLETON and P. EGGLETON. (*Depts. of Materia Medica and Physiology, University of Edinburgh.*)

The effect produced by lack of oxygen on the mechanical response of the frog's isolated ventricle depends on the reaction and on the buffering of the perfusion fluid. With unbuffered Ringer's fluid, lack of oxygen reduces the mechanical response to 10 p.c. of the normal in 20 minutes and produces complete arrest in less than an hour. In an alkaline (pH 8·5) well buffered fluid, lack of oxygen only reduces the response to 80 p.c. of normal in an hour, and arrest occurs after two or three hours.

The normal value of the phosphagen index (phosphagen phosphorus/ortho-phosphate phosphorus) is 0·63, and this is reduced to 0·1 or less in 15 minutes by oxygen lack. This reduction occurs at the same rate and to the same extent in neutral and in alkaline Ringer's fluid. A ventricle perfused with the latter can therefore maintain an almost normal response for about an hour after the phosphagen index has been reduced to less than one-sixth of its normal value. (The sum of the phosphagen phosphorus and the inorganic phosphorus does not alter.)

The addition of alkali will not only delay the depression of mechanical response by oxygen lack, but will restore the activity of a heart arrested by oxygen lack in neutral Ringer's fluid, and such a restoration may persist for nearly an hour. This restoration of mechanical activity is not accompanied by any rise in the phosphagen index, although the latter rises at once when oxygen is readmitted. There is therefore no certain relation between phosphagen content and mechanical activity, except that a small phosphagen content (about 1 mg. per 100 g.) has been found in all hearts that were not irreparably damaged.

Iodo-acetic acid in appropriate concentration (e.g. 1 : 50,000) produces no measurable effect on the mechanical response of the ventricle within two hours, provided oxygen is supplied, but when oxygen is replaced by nitrogen the ventricle is arrested in 5 minutes. The activity of the ventricle can be nearly completely restored by immediate readmission of oxygen, but if the anaerobiosis is prolonged a contracture ensues and this is irreversible.

The changes in the phosphagen index shown by these hearts resemble those observed in unpoisoned hearts, i.e. the index is high as long as oxygen is present, it falls immediately oxygen is removed, and rises when oxygen is readmitted soon enough to permit of recovery of mechanical activity. Unlike the case of skeletal muscle the sum of inorganic and phosphagen phosphorus does not diminish under the action of the drug.

The effects described above can be produced by 40 γ or less of iodo-acetic acid on a ventricle weighing 0·2 g.

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The anticoagulant action of chlorazol. By A. St G. Huggett and H. Silman. (*Dept. of Physiology, University of Leeds.*)

Chlorazol Sky Blue FF is a *bisazo* dye which, under the name of Chicago Blue, was described by Rous, Gilding and Smith [1930] as having an anticoagulant action on intravenous injection. It is in the Colour Index as No. 518. It is characterized by a large molecular weight, 992. It is readily soluble in water after purification by dialysis and recrystallization. An 8 p.c. solution is isotonic with blood and has a pH of 8·1. The anticoagulant dose varies from 1 c.c. per kg. body weight to 5 c.c. per kg. body weight intravenously of the isotonic solution. 1 c.c. per kg. in rabbits, goats or cats prolongs the clotting time to 12 hours or more if the blood is removed within 5 minutes of injection. If the time that elapses after injection is increased, the clotting time becomes shorter, so that it may only be 5 to 7 minutes after 1 hour's interval from the injection of the chlorazol. 2 to 3 c.c. per kg. yield a clotting time of several hours for several hours after injection. The fluid blood obtained from the animal can be clotted *in vitro* by the addition of tissue extract, which suggests that the chlorazol acts as an antithrombokinase. To test this, thrombokinase was prepared from bird testis, fibrinogen-prothrombase complex from bird plasma, and also prothrombase alone from bird plasma by the methods described by Mellanby [1908, 1930]. Calcium was used as 1 p.c. calcium chloride. It was then found that the clotting effect of calcium and thrombokinase on the fibrinogen-prothrombase complex was inhibited by chlorazol. It had no effect, however, on the action of thrombase itself. It therefore inhibits the first stage of blood clotting. It was not incubated with the four components in turn for 10 minutes, and then the mixture added to the other three components. Only with thrombokinase was any evidence of inhibition by chlorazol obtained. This was confirmed by the fact that chlorazolized fluid blood was clotted by thrombase within 1 minute and by excess of thrombokinase within 3 minutes.

It is therefore an antithrombokinase. Its action on the body is to raise the blood-pressure slightly, to improve respiration, and apparently it has little or no effect upon the gas carrying power or buffering action of plasma. It has an advantage over heparine in that it is cheaper and is not antagonized by agitation of the blood in an exterior circulation.

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The relation of different foodstuffs to the alkaline tide of the urine. By C. E. Brunton and C. Wilson. (*Dept. of Physiology, London Hospital.*)

It has been shown [Brunton and Israëls, 1930] that the rise of alveolar CO_2 first described by Dodds [1921] occurs oftener if the secretion is brought about by meals than by drugs. The expression "alkaline tide" should not be applied to samples of urine which merely show a decreased percentage acidity or cH, but should be reserved for a decreased acid output in unit time. This has been recognized by Leathes [1919] and Dodds [1923] but has not been realized by others whose conclusions are therefore useless as a basis for quantitative correlation of the mechanisms for maintenance of the blood reaction.

We have confirmed the occurrence of a morning alkaline tide before the first meal [Leathes, 1919; Taylor, 1930] but do not discuss the phenomenon here. Previous investigators of the alkaline tide have not always included information about the normal variations in their subjects when fasting, or the occurrence of diuresis. These factors might influence the true alkaline tide. For this reason we have studied (1) the output of the subjects' kidneys when no food or liquid was taken, (2) the effect of diuresis, and (3) that of individual foodstuffs on the urine during the 3 hours following their ingestion.

Methods. Urinary acidity was measured by the following methods: (1) titration with 0·1 N. NaOH to phenolphthalein, (2) the pH of the urine by the quinhydrone electrode, and (3) electrotitration to pH 7·4. Method 1 gives a curve similar to Method 3, but with larger acid values in an acid urine and smaller alkaline values in an alkaline urine.

Results. Subjects were examined from 11 a.m. till 3 or 4 p.m. when no

food was taken later than a standard breakfast at 8 a.m. These experiments show that in the subjects studied no diuresis and no decrease in minute acid output, *i.e.* no true alkaline tide, occurs between 12 noon and 3 p.m. No diuresis is produced by drinking less than 200 c.c. of water, so that any smaller water content of food might be expected to have no diuretic effect under the standard conditions. If 375–500 c.c. of water are drunk at noon a diuresis is produced and the titratable acidity tends to fall during the diuresis, but not to such an extent as to prevent the increased volume of urine producing an increase in the minute acid excretion, *i.e.* an acid tide. From a study of the first 3 hours after taking the water it is clear that such quantities of water as the subjects took will not, in their case, produce a true alkaline tide, although the titratable acidity and *c*H of the specimens decrease during the diuresis, the urinary *c*H approaching that of the blood. The effect of diuretic substances is being studied.

It was thought possible that glucose might act as a protein saver, but so far our results are inconclusive.

As regards the acid- or alkali-producing properties of foods it is well known that sodium bicarbonate and sodium citrate will produce a true alkaline tide. Foods containing an excess of alkali radicles in their ash [Sherman and Gettler, 1912] were taken. Contrary to expectation both raisins and prunes, which have a high excess of alkali radicles, produce no alkaline tide but may produce an acid tide. Potatoes and bananas, on the other hand, with a lower alkali excess, produce a true alkaline tide. Evidently the effect of food on the real acid output during the 3 hours following its ingestion does not depend entirely on the relation of acidic and basic radicles as usually estimated. Possible explanations may be (1) differential absorption of different constituents of the food, or (2) metabolic effects after absorption, *e.g.* the oxidation or non-oxidation of organic acids which are lost when the food is ashed *in vitro.*

The suggestion has been brought forward that different foodstuffs have different effects on the secretion of hydrochloric acid by the stomach, thus influencing the alkaline tide [Kaye, 1929]. Bovril meals were given and stomach analyses were made which showed that a good gastric secretion had occurred. The titratable acidity of the urine fell as in Kaye's experiments, but there was no true alkaline tide.

Summary. The moderate water diuresis produced in these experiments, though accompanied by a reduction in the titratable acidity of the urine, was not accompanied by a true alkaline tide.

Acid and alkali tides have been obtained with certain foodstuffs. Neither the acid or alkali content of the ash nor the effect of the food-

stuffs on the hydrochloric acid secretion is adequate to explain these results.

One of us (C.W.) held the Eliza Ann Alston Scholarship during the time the work was being done. To the administrators of this fund we offer sincere thanks.

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The refractory period of the perfused rabbit's ventricle.

By A. S. Dale and A. N. Drury[1].

It has been shown, in the intact dog, that in the rhythmically driven auricle the duration of the refractory period of the auricular muscle shortens as the rate of beating is raised [1921]. Using a method similar to that employed in those observations and testing the period on the epicardial surface of either left or right ventricle, we have been unable to find this relation in the perfused rabbit's heart. The period in such a preparation remains relatively constant over a considerable range of rates, though the duration is usually slightly shorter at high rates than it is at low rates of beating. This result is found both in the whole heart and in strips of ventricular muscle, and whether the preparation is perfused in the usual manner with Ringer's solution, blood Ringer, or whole blood, with or without the addition of atropine.

In the intact atropinized rabbit the period shortens as the rate of beating is raised, and a curve similar to that described for the dog's auricle is obtained. This same result however—namely, shortening of period with rise of rate—is obtained not only in the intact atropinized cat, but also in the perfused cat's heart when tested on its epicardial surface. On the other hand if the refractory period is measured in the perfused cat's heart with the stimulators embedded in the endocardial surface of the ventricle, the refractory period remains relatively constant, though if tested in the same preparation on the epicardial surface it shortens as the rate of beating is raised.

These two different results can also be obtained in the intact atropinized rabbit or cat. Two stimulating electrodes, consisting of straightened fishhooks, are forced, about 2 mm. distant from one another, through the ventricular wall and drawn back till the barbs of the hooks catch the endocardial surface. The hooks are everywhere carefully insulated except on the internal surface of the barbs themselves, so

[1] Working on behalf of the Medical Research Council.

that the ventricle is stimulated from its endocardial surface. Under these conditions the duration of the period is relatively constant over a considerable range of rates, whereas the usual relationship between length of period and rate is obtained from measurements with stimulators embedded in the epicardial surface. The results obtained in the perfused rabbit's heart seem to be due therefore to the altered tissue resistance combined with the relatively thin muscular walls, allowing the testing shocks to reach the endocardial surface of the ventricular chambers. The endocardial surface contains Purkinje tissue, and it is reasonable to conclude that the constant period measurements are therefore of Purkinje tissue and not of the ventricular muscle itself.

In the intact rabbit it is possible to obtain a constant refractory period after (1) removal of both stellate ganglia, or (2) injection of a considerable volume of saline into the external jugular vein. In the latter case the most likely explanation is that the saline alters the tissue resistance and allows the testing shocks to reach the Purkinje fibres. In the former, the removal of sympathetic tone may lead to similar altered tissue conditions or to an actual increase in the excitability of the Purkinje tissue. In the intact cat, removal of both stellate ganglia, and their associated thoracic chains, has no influence. The results show therefore that the perfused rabbit's heart is not a suitable preparation for investigating the refractory period of ventricular muscle. In addition, they supply evidence that the endocardial surface of the ventricular chambers contains a tissue—in all probability Purkinje tissue—whose refractory period is relatively uninfluenced by the rate of beating.

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Observations on the caudal region of the large bowel.

By R. C. Garry. (*Institute of Physiology, the University, Glasgow.*)

In the majority of cases decerebrated or spinal cats were used as also in a few cases cats under sodium amytal anæsthesia. The gut movements and responses were recorded by two tandem balloons introduced through the anus. The distance between the balloons could be varied at will and manipulation of the balloons themselves served as the stimulus. By this means the response of the gut in the neighbourhood of the stimulating balloon or at a distance could be recorded.

The terminal portion of the large bowel and the anal canal were extremely unresponsive to rhythmical or sustained distension but were remarkably sensitive to friction. Both to and fro movement and simple rotation of the stimulating balloon were potent stimuli.

The large bowel contracted locally in response to such stimulation even when completely decentralized by spinal anæsthesia. Local stimulation of the anal canal caused dilation of the anal canal.

In addition, movement in the anal canal caused a rise in pressure or a diminution in volume of the large bowel at a distance, while movement in the large bowel caused dilation of the anal canal. Both these responses were facilitated by section of the lumbar sympathetic outflow and were unaffected by section of the pudendal nerves. Such responses disappeared on applying cocaine to the gut mucous membrane, on inducing spinal anæsthesia and on section of the pelvic nerves.

The cost of animals was defrayed by the Carnegie Trust for the Universities of Scotland.

A note on the viscosity method for measuring percentage volume of cells in blood. By Eric Ponder and George Saslow. (*Washington Square College, New York.*)

We have recently [1930] commented on the defects of the viscosity method, as it was used by Suzuki [1921], for measuring the percentage volume of red cells in blood. There is, however, another way of using viscosity determinations for the same object; this method, tentatively suggested by Lamb [1930], is based on the investigations of Trevan [1918]. The formula used for computing the percentage volume p, is

$$n = 1 + \frac{Kp}{100} \qquad ......(1),$$

in which n is the ratio of the viscosity of whole blood, η_b, to that of the plasma, η_p, and K a constant having the value 6·3. This formula is a rearrangement of the expression arrived at by Einstein [1906, 1920], Hatschek [1920], and by Kermack, McKendrick and Ponder [1929],

$$\eta_b = \eta_p (1 + k\phi) \qquad ......(2),$$

where ϕ is the fraction of unit volume occupied by the disperse phase, and k a constant. According to these formulæ p is a linear function of $(n - 1)$ in expression (1), and ϕ a linear function of $(\eta_b/\eta_p - 1)$ in expression (2).

We have carried out a number of determinations by this method on the blood of various animals, the blood being diluted with its own plasma so that the percentage volume of disperse phase varied from 29·1 to 3·65, and the true value for the percentage volume being found by our colorimetric method [1930]. The viscosity measurements were made in the usual way. The results of these experiments show that the relation between p and $(n-1)$ is not at all linear over the range investigated, but that the curve relating the variables is concave to the $(n-1)$ axis. This result is similar to that obtained by Trevan in the sense that a "straight line" drawn through his experimental points makes an intercept on the p-axis. It is therefore clear that equations (1) and (2) cannot be used to compute percentage volume over any extensive range.

There is every reason, on theoretical grounds, to expect these equations to be inapplicable. The question has been fully discussed in a somewhat different connection by Kermack, McKendrick and Ponder, and, as there explained, the validity of equation (2) is limited to the range $\phi < 0{\cdot}1$ or $p < 10$. Further, it is there shown that the value of the constant k depends on the homogeneity of size of the cells (including rouleaux formation), the rigidity of the cells, and the amount of crenation present. Experimentally it is found that K (in equation (1)) varies from 2·8 to 6·3, which is not surprising. Since the general utility of the method depends on the constancy of K, it is obvious that, in investigations necessitating the accurate determination of percentage volume, its use is impossible.

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A water manometer for class use. By Eric Ponder. (*Washington Square College, New York.*)

Water manometers made with specially shaped floats of glass or celluloid (as that of Masters [1930]) are both fragile and difficult to adjust. A simple and efficient manometer which will stand hard usage can be constructed by making the float of hard rubber tubing such as is used in the construction of radio sets. The internal diameter of the glass

tubing used is about 7 mm.; the float is made of a piece of 6 mm. rubber tubing 4 cm. long, and is open at the bottom. An aluminium wire, with a balanced aluminium writing point at right angles to it, is cemented to the upper end of the float, and this wire passes through a brass cap at the upper end of the open limb of the manometer. The manometer so constructed is in every way as efficient as the more elaborate instrument of Masters, and, because of its simplicity and low cost, is suitable for class use.

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Sensory impulses produced by heat and injury.

By E. D. Adrian.

When the frog's skin is stimulated by gradually increasing pressure the sensory discharge in the cutaneous nerve consists at first of impulses of large action potential travelling rapidly, but later on slow impulses of smaller potential appear as well. These are found whenever the skin is injured and they may continue for some time after the actual infliction of the injury. Records of them have been published elsewhere [Adrian, 1931] with the suggestion that they are due to the nerve fibres responsible for the *C* elevation which Erlanger and Gasser [1930] find in the potential wave of a nerve trunk stimulated electrically. This suggestion was made with the knowledge that the rate of conduction of the *C* wave (0·6–0·3 metres per sec. in the frog) is considerably slower than that of many of the impulses produced by injury: but the impulses could not be definitely ascribed to the nerve fibres which give the *B* wave (4–1 metres per sec.) for Erlanger and Gasser were unable to find this in the sensory roots of the cord.

More accurate measurements of conduction velocity have now been made on the ventral and dorsal branches of the sixth and seventh spinal nerves and on some of the nerves of the leg. The velocity of the impulses produced by damaging the skin varies widely and covers a range of 4·0 to 0·5 metres per sec. In a typical record the majority of the slow impulses travel at rates between 3 and 1·5 metres per sec. In the same preparations the larger impulses set up by light touch travel at 18–15 metres per sec., and there are occasional smaller impulses travelling as slowly as 10 metres per sec. (All measurements at 14–17° C.)

Impulses with a velocity intermediate between that of the rapid and the slow group are produced by stimulating the skin by heat. A coil of

platinum wire heated electrically and held a few mm. from the skin will eventually produce slow impulses indistinguishable from those due to mechanical injury, but before these appear there is often a discharge of impulses with velocities ranging from 9 to 4 metres per sec. These impulses are smaller than the tactile impulses and larger than those due to injury; they have not been found with any form of mechanical stimulation.

Thus the sensory impulses from the frog's skin travel with velocities which cover the whole range between 18 and 0·5 metres per sec., and there is no distinct gap corresponding to that between Erlanger and Gasser's *A* and *C* waves. Making allowances for differences of temperature etc. it appears that the impulses due to injury mostly travel with velocities characteristic of the *B* wave. It is possible, in fact, that impulses corresponding to the *C* wave are too small to appear in records made from peripheral nerves of medium size, for many slower and smaller impulses can be detected in the sensory roots where the conditions for recording are more favourable.

Erlanger and Gasser have found the *B* wave in the gray rami, but as no impulses of this velocity are found in efferent sympathetic discharges we must conclude that the *B* fibres are mainly, if not entirely, sensory. The recent work of Kuntz and Farnsworth [1931] shows that some sensory fibres pass up the sympathetic chain before they enter the cord. It is possible that this may account for the absence of the *B* wave in the sensory roots examined by Erlanger and Gasser, but in the frog (*Rana Temporaria*) some other explanation must be sought, for in all the sensory roots examined the impulses from the skin seem to vary over as wide a range as they do in the peripheral nerves, though their velocity has not been accurately measured.

It may be noted that the magnitude of the potential change (monophasic) is roughly proportional to the velocity of conduction: if the latter varies directly with the diameter of the fibre, the potential must vary with the diameter rather than the cross-sectional area. In general, however, these results support the conclusion reached by Erlanger and Gasser, namely that impulses from tactile, temperature and pain receptors travel at fast, medium and slow velocity.

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